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(54) Title: METHOD FOR THE INTRANASAL ADMINISTRATION OF L-DOPA PRODRUGS (57) Abstract The esterification of the carboxylic group of L-dopa resulted in derivatives (prodrugs) that are considerably more water soluble and more lipophilic than L-dopa. The esters were found a) to be absorbed rapidly from the nasal cavity of the rat, b) to be rapidly hydrolyzed to L-dopa in rat plasma, brain homogenate and cerebrospinal fluid (CSF), c) to be eliminated after nasal and intravenous administration at a rate corresponding to that of the L-dopa ($t_{1/2}$ = 63.0 minutes), and d) to be relatively stable in aqueous solutions especially at pH's below 5.0. Administration of the L-dopa ester prodrugs intranasally in humans minimizes the peripheral side effects associated with oral L-dopa administration. Since the nasal administration does not result in significant formation of dopamine in peripheral circulation, and this route of administration delivers L-dopa effectively to the blood stream, the utilization of water soluble prodrugs of L-dopa via the nasal route has therapeutic advantages in the treatment of Parkinson's disease.		

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METHOD FOR THE INTRANASAL ADMINISTRATION OF L-DOPA PRODRUGS**FIELD OF THE INVENTION**

5 This invention relates generally to a method for enhancing the delivery of L-dopa to the brain of a mammal in need of treatment with this drug, by administering water-soluble prodrugs of L-dopa intranasally. More specifically, this invention relates to the enhancement of L-dopa treatment by intranasal administration of water-soluble esters of L-dopa. The invention is particularly
10 useful in the treatment of Parkinson's disease.

BACKGROUND

 Idiopathic Parkinson's disease was first described by James Parkinson in 1817 as *paralysis agitans*. Parkinsonism is a clinical syndrome composed of four features: bradykinesia, muscular rigidity, resting tremor, and abnormalities of
15 posture and gait. This disorder results from damage to the basal ganglia of the brain, particularly the substantia nigra. In the healthy state, nervous signals pass from the brain's cortex through the reticular formation and spinal cord to muscles. A negative-feedback signal passes to the basal ganglia via a second pathway, producing a damping effect on the corticospinal pathway. This
20 feedback signal reduces muscle tone, resulting in smooth, jerk-free muscle control during movement. Dopamine, a neural transmitter produced by the substantia nigra, is primarily responsible for producing this damping effect. Another transmitter, acetylcholine, inhibits the damping effect. A balance is maintained between dopamine and acetylcholine in the healthy state.

25 In Parkinson's disease, as the result of degeneration of the basal ganglia, dopamine activity is decreased, while acetylcholine activity remains. As a result, the muscles are over-tense, causing tremor, joint rigidity, and slow movement (bradykinesia). Most drug treatments for Parkinson's are based on either

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increasing the level of dopamine in the brain or neutralizing the action of acetylcholine.

The symptoms of Parkinson's disease usually become manifest after the age of 55, and increase after the age of 65. Left untreated, Parkinsonian patients become rigid and akinetic, and require constant care. Death is usually due to the complications of pulmonary embolism, aspiration, or hypostatic pneumonia. In spite of advanced understanding of its pathophysiology and treatment, the root causes of Parkinsonism remain unknown.

Amelioration of dopaminergic transmission restores motor function in Parkinsonism. This amelioration forms the central strategy of almost all current drug regimens for the treatment of this disease. However, systemic administration of dopamine does not result in higher brain dopamine levels, because dopamine cannot cross the blood-brain barrier. On the other hand, its precursor, L-dopa, can cross the blood-brain barrier. Once in the brain, L-dopa is metabolized (decarboxylated) to form dopamine. L-dopa is currently considered the first-line therapy for the management of Parkinson's syndrome¹. However, as discussed below, there are significant problems associated with its current administration.

L-dopa is L-3,4-dihydroxyphenylalanine (Figure 1). It is an odorless white to off-white crystalline powder which melts with decomposition at 270°C. The approximate solubility of L-dopa at 25°C in different solvents is listed in Table 1.

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Table 1: Solubility data for L-dopa⁹.

Solvent	Solubility (mg/ml)
Water	1.65
95% Ethanol	0.30
5 Methanol	0.10
2-Propanol	<0.01
Chloroform	0.10
Diethyl Ether	<0.01
Propylene Glycol	0.20
10 Acetone	0.03
Acetonitrile	0.07

In water, L-dopa exists in different ionic forms depending on the pH of the solutions. At a range of pH from 3 to 9, L-dopa exists as a zwitterion. This contributes to its low water solubility in this pH range, as shown in Figure 2¹⁰.

15 As noted above, L-dopa represents the most clinically useful drug in the treatment of Parkinson's disease, because unlike dopamine, L-dopa crosses the blood-brain barrier and is converted to dopamine in the brain. The magnitude of improvement in Parkinsonism with L-dopa therapy has not been surpassed by any other available anti-Parkinsonian agent³.

20 L-dopa is typically administered orally in large doses, either by itself (i.e., Larodopa[®]) or in combination with a decarboxylase inhibitor (i.e. Sinemet[®]). Unfortunately, the clinical response to oral L-dopa is variable and unreliable because of its erratic oral absorption and first-pass metabolism. The oral bioavailability of L-dopa administered alone is estimated to be only about 5
25 to 10%, and only 3% of the administered oral dose actually reaches the brain.

Several factors are believed to be responsible for the inefficiency of the oral dosage forms. First, L-dopa undergoes carrier-mediated active transport absorption in the intestine¹⁴. It has been shown, in studies carried out in isolated dog intestinal segments *in situ*, that the major absorption site for L-dopa is the

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duodenum¹¹. The extent of absorption decreases after the drug passes the upper part of the small intestine. Figure 3 shows that the duodenal segment of the dog intestine is the most efficient absorption site. Such site-specific absorption limits the extent of absorption of orally administered L-dopa.

5 Second, L-dopa undergoes extensive metabolism in the gastrointestinal (GI) wall during the absorption process. As shown in Figure 4, the plasma levels of L-dopa after intravenous and hepatic portal infusions in dogs are identical, whereas the plasma level after duodenal administration is extremely low¹⁶.

10 Metabolism of L-dopa in the GI wall appears to be dose-dependent. The area under the blood level curve increases disproportionately as a function of the oral dose, possibly due to a saturable metabolic process¹¹. The oral bioavailability of L-dopa without decarboxylase inhibitors is 15% at a dose of 3.8 mg/kg/day and 33% at a dose of 15.4 mg/kg/day. At higher doses, the enzymes
15 become saturated, resulting in disproportionately higher bioavailability.

 L-dopa is metabolized to several products (Figure 5)¹³, some of which have their own pharmacological activities and side effects. The metabolism of L-dopa occurs mainly by decarboxylation and conjugation in the gastrointestinal tract before entering the systemic circulation. One major pathway for the
20 metabolism of L-dopa is its decarboxylation to dopamine (Figure 6)¹⁴.

 It is believed that the major peripheral side-effects resulting from the oral administration of L-dopa are due to the formation of large amounts of dopamine during first-pass metabolism in the GI wall. These side-effects include nausea, vomiting and cardiac irregularity. Thus, the lowest possible dose of L-dopa
25 which can be administered is desired because of the undesirable systemic side effects.

 Inter- and intraindividual variability in the degree of this first-pass effect is the main cause of the common difficulty of maintaining an effective therapeutic regimen with L-dopa. In order to reduce L-dopa doses and reduce
30 the side effects, decarboxylase inhibitors may be coadministered with L-dopa.

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The most notable effect of this co-administration is a 75% reduction in total daily L-dopa dose required to produce clinical benefit^{13,15}. The oral bioavailability of L-dopa is doubled by coadministration of dopa decarboxylase inhibitors¹⁶, and C_{max} (maximum concentration) and AUC (Area Under the Curve) for a given dose are also increased¹⁷. The utilization of such inhibitors also decreases peripheral side effects; however, abnormal involuntary muscle movements and adverse mental effects tend to develop earlier in therapy. The on-off fluctuation remains, because the oral absorption is still erratic and plasma concentrations still fluctuate. Thus, the oral route of administration has significant drawbacks.

On the other hand, intravenous infusion of L-dopa, either alone or in combination with a decarboxylase inhibitor, dramatically extends the duration of mobility, reduces the frequency of fluctuation, and provides significant mobility improvement¹⁸⁻²². Intravenous infusion provides constant plasma levels of L-dopa, leading to better management and more consistent symptomatic control of the disease. Kurlan et al.²³ have compared plasma L-dopa profiles obtained by administering identical doses of L-dopa and carbidopa as standard Sinemet® (25 mg carbidopa/100 mg L-dopa), by continuous instillation of an equivalent dose into the stomach, and by continuous intraduodenal and intragastric infusion (Figure 7). The most uniform plasma L-dopa levels and clinical responses were obtained with continuous intraduodenal infusion. Intermittent infusion of 100 mg of L-dopa into the duodenum provided regular and reproducible peaks of plasma L-dopa and motor activity. Continuous or intermittent gastric infusion mimicked the pattern of plasma L-dopa and clinical response seen with oral administration of standard Sinemet® 25/100. This further proves that gastric emptying contributes to the fluctuation of plasma L-dopa levels and results in fluctuating responses after oral-absorption. Thus, intravenous infusion offers significant advantages over the oral route of administration.

Intravenous infusion, however, is impractical and inconvenient for routine clinical use because of the large volumes of fluid required and the acidity of L-dopa solutions, let alone the fact that patients would rather not have to inject

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themselves each day in order to get the desired clinical effect. Attempts to enhance the bioavailability and minimize the side effects of L-dopa administration include modifications in the formulation of L-dopa-containing pharmaceutical compositions, and utilization of prodrugs of L-dopa orally and rectally. While
5 none of these approaches have overcome the difficulties with L-dopa, nevertheless, a brief description of each attempt is discussed below.

The concept of retaining an oral formulation, while delaying release of the active substance until the formulation reaches the upper part of the small intestine has been considered. Since L-dopa is absorbed from the upper part of the
10 intestine, and since its GI metabolism is a saturable process, Sasahara et al.¹² proposed an oral tablet formulation that would release most of the drug in the upper part of the intestine and thus provide a very high concentration at the site of absorption. The design of their dosage form was accomplished by using polymeric materials that resist the stomach environment but open rapidly in the
15 upper part of the intestine. While this formulation may increase bioavailability, it is subject to significant influence by factors such as stomach emptying time, GI transient time, and other uncontrollable physiological factors. This may result in high variation between patients. Thus, the super enteric coated tablet envisioned by Sasahara et al. has not received wide acceptance for clinical use in
20 Parkinsonism.

Other oral formulations employing controlled-release techniques have also been considered. L-dopa is absorbed from the upper-most part of the small intestine. As a result, the drug is a poor candidate for incorporation into a sustained release oral dosage form²⁷. A study conducted by Dempsey et al.¹³,
25 comparing the bioavailability in humans of two formulations of Sinemet® with two different release rates showed that the faster the rate of release the greater the bioavailability. As shown in Figure 8, formula CR4, which had a $t_{1/2}$ (half-life) of release of 0.75 hours, resulted in greater bioavailability than formula CR3, which had a $t_{1/2}$ of release of 2.5 hours.

30 The bioavailability for oral extended-release Sinemet® CR4 tablets is

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about 70-75% of that for standard Sinemet® tablets. To compensate for the differences, the total dose for patients taking Sinemet® CR4 tablets is about 25% more than that for the standard Sinemet® formulation^{24,25}. Sinemet® CR4 (50/200) allowed a slight extension in the interval between doses as compared
5 with standard Sinemet®.

Cedarbaum et al.²⁶ compared the clinical effectiveness and plasma levels of L-dopa following oral administration of standard Sinemet® tablets and Sinemet® CR4 tablets in Parkinsonian patients (Figure 9). Although the extended-release tablet regimen increased the total "on" time and decreased the
10 total "off" time, compared with the standard preparation, there were still fluctuation in both plasma L-dopa levels and clinical responses, despite the fact that higher doses of the extended-release tablets were used.

The controlled released Sinemet® CR4 can reduce but not eliminate fluctuations in response. The application of this technology will continue to be
15 limited by factors such as erratic gastric emptying time. Overall, the Sinemet® CR4 is not an ideal formulation, and there are significant problems associated with its use. In particular, the slow rise and fall of plasma L-dopa levels cause successive doses to contribute to progressively higher levels of the drug late in the day, in turn causing prolonged and at times severe dyskinesia. The increased
20 lag time to the onset of clinical effect related to the slow rise in plasma L-dopa levels ($t_{\max} = 1.9$ h versus 1.2 h for standard Sinemet®) proved inconvenient for some patients. Thus, this was compensated for by the addition of standard release Sinemet® to the treatment program²⁷.

Another approach to achieving improved bioavailability of L-dopa is to
25 modify L-dopa structurally²⁸ and possibly select a route of administration other than the oral route. The transient modification is intended to (a) increase water solubility, (b) increase lipid solubility, and (c) protect the drug from enzymatic inactivation. Most of the chemical modifications involve the esterification of the catechol or the carboxylic acid moieties. The conversion of these prodrugs to the
30 parent compound by widely distributed esterases makes such structural

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modification very attractive.

For example, the methyl ester of L-dopa given intraperitoneally was found by Hanson and Bennett to be equivalent to L-dopa in reversing the effects of reserpine in cats^{20,29}. Unfortunately, the oral administration of these prodrugs to dogs resulted in only a marginal improvement (by a factor of 2) in the bioavailability of L-dopa³⁰. It was concluded that oral administration of L-dopa prodrugs did not eliminate the problems associated with oral administration of L-dopa itself.

Cooper et al.³¹ have shown in animal studies that oral administration of a series of L-dopa esters results in behavioral activity that is not markedly different from that observed after oral administration of L-dopa itself. These results likely reflect rapid hydrolysis of the L-dopa esters to L-dopa in the small intestine. Once L-dopa is released from these prodrugs, it is subjected to metabolic inactivation to dopamine or other metabolites in the intestine. Therefore, the esters elicit behavioral responses similar to L-dopa. The search for the preferred method and route of administration continued.

It was determined that the rectal route of administration of the carboxylic acid ester of L-dopa does not offer any advantages over L-dopa. In the rectal cavity, where enzymatic activity is apparently less significant, the limiting factor is the permeability of the rectal mucosa to the esters. Once the esters reach the plasma compartment, *in vivo* conversion to the parent drug can occur. Fix et al.³² have shown that rectal administration of alkyl esters of L-dopa with carbidopa in dogs resulted in bioavailabilities ranging from 7 to 51%. None of the esters afforded systemic L-dopa bioavailability comparable to intravenous administration of L-dopa itself. The usefulness of these prodrugs for oral or rectal administration is thus very limited.

Because of the above, a long felt need exists in the art for an alternative and improved method for the utilization of L-dopa in Parkinson's patients.

Recently, the nasal route of administration has received a great deal of attention as a convenient and reliable method for the administration of drugs, and

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serves as an alternative to intravenous administration. For example, butorphanol tartrate (Stadol NSTM) is ineffective orally but is commercially available in the form of a nasal spray.

It has also been demonstrated that the rate and extent of absorption of
5 drugs from the rat nasal cavity matches that in humans. For example, the nasal absorption of propranolol^{5,1,7} is identical in both rats and human. Figures 10 and 11 show plasma levels for propranolol in rats and humans^{6,7}. Based on the above, the rat is a good model for studying the nasal absorption of L-dopa and its prodrugs.

10 Surprisingly, the present inventors have found that the nasal route of administration of water-soluble prodrug of L-dopa offers significant advantages over the prior art. Those advantages include almost 100% bioavailability, no dopamine in the plasma thereby avoiding undesirable systemic side effects and a much larger dose actually reaching the brain to achieve the desired clinical
15 effect.

In view of the foregoing, it is apparent that there exists a need in the art for improved methods of delivery of L-dopa for treatment of Parkinson's disease and other disorders characterized by decreased dopamine.

SUMMARY OF THE INVENTION

20 Accordingly, since L-dopa is too insoluble to be used in conventional intranasal formulations, and since water-soluble prodrugs of L-dopa are ineffective when administered orally or rectally, it is an object of the present invention to provide a method for treating dopamine deficiency comprising intranasal administration of water soluble prodrugs of L-dopa.

25 It is a further aspect of this invention to provide a method for administering L-dopa in a manner which significantly enhances plasma levels of L-dopa, and thus its bioavailability, compared to prior art methods. The present

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inventors have found that while prior art methods provide for 3-4% bioavailability, intranasal administration of water soluble prodrugs of L-dopa of the present invention provides for 10% or greater bioavailability of L-dopa.

It is a further aspect of this invention to provide a method for
5 administering L-dopa which provides for enhanced delivery of dopamine directly to the brain. In the present invention, when water-soluble prodrugs of L-dopa are administered intranasally, as much as 3% of the administered dose is absorbed directly into the central nervous system, its intended site of action.

It is a still further aspect of this invention to provide a method for
10 administering L-dopa which does not appreciably elevate dopamine levels in the peripheral circulation, thus minimizing the side-effects associated with conventional L-dopa administration. This object has been achieved in the present invention by the nasal administration of water-soluble esters of L-dopa.

It is a still further aspect of this invention to provide a method for
15 administering L-dopa which is equal or superior to intravenous administration in many respects, including effectiveness, but which avoids many of the problems associated with the intravenous route, including the "on-off effect," combined with superior ease of administration.

A further aspect of this invention is to provide a pharmaceutical
20 composition suitable for intranasal administration, for treatment of dopamine deficiency, including Parkinson's disease. Accordingly, the composition of the present invention comprises a water-soluble prodrug of L-dopa and a pharmaceutically acceptable carrier.

With the foregoing and other objects, advantages and features of the
25 invention that will become hereinafter apparent, the nature of the invention may be more clearly understood by reference to the following detailed description of the preferred embodiments of the invention and to the appended claims.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Chemical structure of L-dopa.

Figure 2: pH-solubility profile for L-dopa at 37°C.

Figure 3: Average AUC of L-dopa up to 1 hour after administration of single
5 100 mg dose of L-dopa to dog duodenum, jejunum, and ileum.

Figure 4: Average plasma levels of L-dopa following three routes of
administration of single 20 mg dose of L-dopa to dogs.

Figure 5: Major metabolic pathways of L-dopa.

Figure 6: Average (\pm SE) plasma levels of L-dopa (A) and total dopamine (B)
10 following oral administration of L-dopa to three patients. Key: \blacktriangle , 3.8
mg/kg; \circ , 7.7 mg/kg; and \bullet , 15.4 mg/kg

Figure 7: Plasma L-dopa levels (filled symbols and left-hand scales) and clinical
performance (open symbols and right-hand scales) in a patient with
response fluctuations during administration of standard Sinemet® tablets
15 (25/100)(A), intermittent duodenal (B), continuous gastric infusion (C),
and duodenal infusion (D) of L-dopa; dots at the top of graphs A, and B,
denote times of drug administration. \bullet , plasma L-dopa; \circ , mobility

Figure 8: A comparison of *in vivo* plasma levels of L-dopa following
administration of CR3 and CR4 tablets.

20 Figure 9: Parkinson mobility scores (right panel) and plasma L-dopa levels for a
typical patient taking standard Sinemet® (open symbols and dashed lines)

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every 3 hours, and Sinemet® CR4 (close symbols) every 6 hours.

Figure 10: Time course of the average blood propranolol levels in three rats following nasal administration of 1 mg/rat (○), intravenous administration of 1 mg/rat (▲), oral administration of 1 mg/rat (●), and nasal administration of 2 mg/rat (□).

Figure 11: Time course of the average serum propranolol levels in six male subjects following nasal administration of 10 mg/subject (▲), intravenous administration of 10 mg/subject (○), and oral administration of 80 mg/subject (□).

Figure 12: *In-vivo* rat nasal operation.

Figure 13: Effect of buffer concentrations on the degradation rate constants of L-dopa butyl ester at 37°C.

Figure 14: pH-rate profiles for the butyl ester at 37°C.

Figure 15: The degradation of the butyl ester in rat plasma.

Figure 16: The degradation of the butyl ester in rat brain homogenate.

Figure 17: The degradation of the butyl ester in rat CSF (cerebrospinal fluid).

Figure 18: The degradation of the butyl ester in the rat nasal perfusate.

Figure 19: The nasal absorption profiles of esters and L-dopa at 4 mg/kg L-dopa equivalent dose. (n=3 for L-dopa, n=4 for Methyl Ester, n=11 for

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Butyl Ester, n=2 for Pentyl Ester, n=2 for Cyclohexyl Ester, n=2 for Benzyl Ester)..

Figure 20: The nasal absorption profiles of the butyl ester at 4, 20, 40 mg/kg L-dopa equivalent doses. (n=3 at 40 mg/kg dose, n=11 at 20 mg/kg dose, n=11 at 4 mg/kg dose).

Figure 21: Plasma L-dopa levels following nasal and intravenous administrations of L-dopa butyl ester at 20 mg/kg L-dopa equivalent dose. (n=11 following nasal route, n=4 following iv route).

Figure 22: L-dopa and dopamine levels following nasal and intravenous administrations of L-dopa butyl ester at 20 mg/kg L-dopa equivalent dose. (n=11 following nasal route, n=4 following iv route)

Figure 23: Plasma dopamine levels following nasal and intravenous administrations of dopamine at 20 mg/kg dose. (n=3 following nasal route, n=2 following iv route).

Figure 24: CSF L-dopa levels following nasal and intravenous administrations of L-dopa butyl ester at 20 mg/kg L-dopa equivalent dose. (n=3 at each point following nasal route except n=2 at 120 minutes, n=1 at each point following iv route).

Figure 25: Olfactory bulb L-dopa levels following nasal and intravenous administrations of L-dopa butyl ester at 20 mg/kg L-dopa equivalent dose. (n=3 at each point following nasal route except n=2 at 120 minutes, n=1 at each point following iv route).

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Figure 26: Rate constants for the absorption, hydrolysis, and the metabolism of L-dopa butyl ester in rats.

Figure 27: Experimental and calculated plasma levels for L-dopa in rat plasma.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

5

Thus, the present inventors have discovered a new and novel method for the treatment of dopamine deficiency, by the intranasal administration of a water-soluble prodrug of L-dopa. This method offers significant clinical advantages over the prior art. More specifically, the inventors sought to provide a safe,
10 effective and convenient treatment for Parkinson's disease which comprises the administration of water-soluble prodrugs of L-dopa intranasally, thus avoiding the side-effects associated with oral dosage forms.

A prodrug is a compound formed by chemical modification of a biologically active compound which will liberate the active compound *in vivo* by
15 enzymatic or hydrolytic cleavage. Advantages of this approach include reduction of general cytotoxicity, better bioavailability of active drug or longer duration of action. Any water soluble prodrug of L-dopa is useful in the practice of this present invention.

The inventors have found that intranasal administration of esters of
20 L-dopa, i.e., prodrugs of L-dopa, effectively ameliorates L-dopa deficiency and are particularly preferred in the practice of the present invention. Intranasal administration of these compounds is as effective as intravenous administration of L-dopa, but may be conveniently and painlessly self-administered by the patient.

Preferred L-dopa esters include alkyl, cycloalkyl, and aryl esters,
25 particularly methyl, butyl, pentyl, cyclohexyl, and benzyl esters, and pharmaceutically acceptable salts thereof.

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Pharmaceutically acceptable salts of an acid group or an amino group include, but are not limited to, salts of organic carboxylic acids such as acetic, lactic, tartaric, malic, isothionic, lactobionic and succinic acids; organic sulfonic acids such as methanesulfonic, ethanesulfonic, benzenesulfonic and *p*-tolylsulfonic acids, and inorganic acids such as hydrochloric, sulfuric, phosphoric and sulfamic acids.

A still further aspect of this invention is a pharmaceutical composition of matter for treating dopamine deficiency that comprises at least one L-dopa ester as described above, mixtures of L-dopa esters thereof, and/or pharmaceutical salts thereof, and pharmaceutically acceptable carriers therefor. Such compositions are prepared in accordance with accepted pharmaceutical procedures, for example, as described in *Remington's Pharmaceutical Sciences*, seventeenth edition, ed. Alfonso R. Gennaro, Mack Publishing Company, Easton, Pennsylvania, Eighteenth edition (1990).

For therapeutic use in a method of treating dopamine deficiency, an L-dopa ester, or its salt, can be conveniently administered in the form of a pharmaceutical composition containing an L-dopa ester, or its salt, and a pharmaceutically acceptable carrier therefor. Suitable carriers are well known to those skilled in the art and vary with the desired form and mode of administration of the pharmaceutical composition. Typically, the carrier may be a liquid, suspension, semi-solid, or vaporizable carrier, or combinations thereof. In a preferred embodiment, the carrier is a pharmaceutically acceptable aqueous carrier.

The compound of the invention or its salt may be formulated together with the carrier into any desired unit dosage form. Unit dosage forms such as solutions, suspensions, and water-miscible semisolids are particularly preferred.

Each carrier must be "acceptable" in the sense of being compatible with the other ingredients in the formulation and not injurious to the patient. The carrier must be biologically acceptable and inert, i.e., it must permit the body's metabolic reactions to effectively transform the esters of this invention into

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dopamine. To prepare formulations suitable for intranasal administration, solutions and suspensions are sterilized and are preferably isotonic to blood.

The formulations may conveniently be presented in unit dosage form and may be prepared by any method known in the art. Such methods include the
5 step of bringing the active ingredient into association with the carrier which itself may encompass one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product. Various unit dose and multidose containers, e.g.,
10 sealed ampules and vials, may be used, as is well known in the art.

In addition to the ingredients particularly mentioned above, the formulations of this invention may also include other agents conventional in the art for this type of pharmaceutical formulation.

Also part of this invention is a method of treating dopamine deficiency,
15 particularly that associated with Parkinson's disease, in a mammal, e.g. human, by treating that mammal with an effective amount of an L-dopa ester intranasally. In this application patient will encompass any mammal suffering from dopamine deficiency, particularly a mammal suffering from Parkinson's disease, or a mammal suffering damage to the substantia nigra of the brain and
20 needing treatment.

The dosage of the L-dopa esters, pharmaceutically acceptable salts thereof, or mixtures thereof, in the compositions of the invention administered to a patient will vary depending on several factors, including, but not limited to, the age, weight, and species of the patient, the general health of the patient, the
25 severity of the symptoms, whether the composition is being administered alone or in combination with other agents, the incidence of side effects and the like. The desired dose may be administered as 1 to 6 or more subdoses administered at appropriate intervals throughout the day. The compounds may be administered repeatedly over a period of months or years, or it may be slowly and constantly
30 infused to the patient. Higher and lower doses may also be administered.

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The daily dose may be adjusted taking into account, for example, the above-identified variety of parameters. Typically, the present compositions may be administered in an amount of about 0.1 to 1.0 mg/kg body weight/day. However, other amounts may also be administered.

5 To achieve good plasma concentrations, the active compounds may be administered, for instance, by intranasal administration of an approximate 0.1 to 1M solution of the active ingredient, optionally in saline.

While it is possible for the active ingredient to be administered alone, it is preferably present as a pharmaceutical formulation. The formulations of the present invention comprise at least one active ingredient, as defined above,
10 together with one or more acceptable carriers thereof and optionally other therapeutic agents.

The above method may be practiced by administration of the compounds by themselves or in a combination with other active ingredients in a
15 pharmaceutical composition. Other therapeutic agents suitable for use herein are any compatible drugs that are effective by the same or other mechanisms for the intended purpose, or drugs that are complementary to those of the present agents.

The compounds utilized in combination therapy may be administered simultaneously, in either separate or combined formulations, or at different times
20 than the present compounds, e.g., sequentially, such that a combined effect is achieved. The amounts and regime of administration will be adjusted by the practitioner, by preferably initially lowering their standard doses and then titrating the results obtained. The therapeutic method of the invention may be used in conjunction with other therapies as determined by the practitioner.

25 Having now generally described this invention, the same will be better understood by reference to certain specific examples, which are included herein for purposes of illustration only and are not intended to be limiting of the invention or any embodiment thereof, unless so specified.

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EXAMPLE 1: General Method of Synthesis of L-dopa Esters

Typically, the prodrug esters of L-dopa may be prepared using a modification of the procedure reported by Patel and Price³⁵. One hundred ml of the appropriate alcohol are placed in a 200-ml three-necked flask equipped with a reflux condenser. The alcohol was cooled to -10°C and nitrogen was bubbled through for 10 min. Thionyl chloride (15 ml) was then added slowly over 15 min, and the reaction mixture was stirred for an additional 15 min. After stirring, 4 g of L-dopa was added, and the mixture was refluxed at 60°C for 12 hr. The ester hydrochloride was precipitated by adding enough petroleum ether to make the solution turbid and then placing the mixture in a refrigerator (4°C) overnight. The final product was collected by filtration and was recrystallized from an acetone-petroleum ether mixture. The crystals were dried in a vacuum desiccator at room temperature and stored in a desiccator until used. The structure and purity of each ester hydrochloride of L-dopa was confirmed by NMR spectra, HPLC, melting point, and elemental analysis. Examples of esters synthesized using this procedure may be found in Table 2, below.

EXAMPLE 2: Stability and Physicochemical Properties of the Ester Prodrugs in Aqueous Buffers*Analytical Procedures*

High-performance liquid chromatography (HPLC) was used for determining physicochemical properties (i.e., chemical stability, partition coefficient etc.). All samples were run at ambient temperature. The resulting chromatograms were recorded on an integrator. Each chromatogram was accompanied by a printout of the peak area, retention time, and the percentage of the total area of each peak.

Analytical Methodology

To determine physicochemical properties (i.e., chemical stability, partition coefficient, etc.), the following HPLC system was used: Beckman 110B Solvent Delivery Module, Spectroflow 757 Absorbance Detector, Spectra-Physics
5 DataJet Integrator, Waters 712 WISP Autoinjector, Waters Nova-Pak C₈ column (3.9 mm x 150 mm).

The mobile phase consisted of 0.05M phosphate buffer at pH 4.0 and acetonitrile. The acetonitrile portion was adjusted according to the ester (see below). The flow rate was set at 1.0 ml/min. The UV wavelength was set at
10 280nm. For L-dopa and its methyl ester, the portion of acetonitrile was 0. The retention time was 1.6 minutes for L-dopa and 11.5 minutes for the methyl ester. For other esters, the portion of acetonitrile was 25%. The retention time was 7 minutes for the butyl ester, 17 minutes for the pentyl ester, 11 minutes for the benzyl ester, and 15 minutes for the cyclohexyl ester.

15 The reactions were initiated by preparing 0.2 mg/ml solutions of the butyl ester prodrug in 0.05M, 0.20M, and 0.50M phosphate buffers at pHs 3.5, 5.5, and 7.4. The solution was kept in screw-capped culture tubes at 20°C and 37°C. At appropriate time intervals, samples were taken and kept on ice until analysis. The other prodrugs were studied in pH 7.4, 0.05M phosphate buffer at 37°C.

20 The rate of hydrolysis of each ester was determined from the slope of the linear plot of the logarithm of the residual ester concentration against time. The experiments were run at least in triplicate for each ester. The pH was determined after each experiment.

The rate constants were calculated and the activation energy was obtained.
25 The pH of optimum stability and the shelf-life at that pH was calculated.

Partition Coefficients

The apparent partition coefficient of each ester was determined at room temperature (20°C) between 1-octanol and pH 7.4, 0.05M phosphate buffer. The phosphate buffer and octanol were presaturated with one another before use

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to minimize the volume change due to mutual solubility. An aqueous phase (5 ml) containing 0.4 mg/ml ester prodrug solution was mixed with 5 ml of 1-octanol. The mixture was manually shaken for 2 min followed by mechanical shaking at 20°C for 1 hour to ensure equilibrium. After centrifugation, the ester concentration in the aqueous phase was measured by HPLC. The partition coefficient was calculated by subtracting the final aqueous phase concentration from the initial aqueous phase concentration to calculate the final octanol phase concentration. The partition coefficient was then calculated by dividing the final aqueous phase concentration into the final octanol phase concentration.

10 *Solubility*

An excess amount of L-dopa and the esters of L-dopa were equilibrated with pH 7.4, 0.05M phosphate buffer in screw-cap vials with constant shaking, vortexing, and sonicating for about one hour. The saturated solutions were filtered through 0.2 µm filter, and the filtrates were analyzed by HPLC.

15 *Results*

Physicochemical Properties

Table 2 lists the physicochemical properties of L-dopa, L-dopa prodrugs, and dopamine. The partition coefficients were measured between octanol and pH 7.4, 0.05M phosphate buffer at 20°C.

Table 2: Physicochemical properties of L-dopa, L-dopa prodrugs, and dopamine

Compound	Formula	Molecular weight	Melting Point (°C)	Partition Coefficient	Solubility (mg/ml)
L-dopa	$C_9H_{11}NO_4$	197.19	276-278	0.01	1.65
methyl ester	$C_{10}H_{14}NO_4Cl$	247.68	170-172	0.25	750
butyl ester	$C_{13}H_{20}NO_4Cl$	289.76	134-137	7.17	660
pentyl ester	$C_{14}H_{22}NO_4Cl$	303.85	143-146	31.56	31
cyclohexyl ester	$C_{15}H_{22}NO_4Cl$	315.79	189-191	25.05	17
benzyl ester	$C_{16}H_{18}NO_4Cl$	323.77	190-192	11.57	5
dopamine	$C_8H_{12}NO_2Cl$	189.64	240-241	0.01	250

Partition coefficient was measured at 20°C, octanol/pH 7.4, 0.05M phosphate buffer. Solubility was measured in pH 7.4, 0.05M phosphate buffer at 20°C.

As shown in Table 2, the prodrugs are significantly more soluble and more lipophilic than L-dopa itself. Based on the desirable physicochemical properties of the butyl ester, this compound was chosen for the nasal absorption studies.

Chemical Stability of the Prodrugs

The hydrolysis of the esters in aqueous solution followed first-order kinetics. The half-lives of hydrolysis for all the esters in 0.05M phosphate buffer at 37°C in pH 7.4, ionic strength adjusted to 1.0 with sodium chloride are shown in Table 3.

Table 3: Half-lives for the degradation of L-dopa esters in 0.05M phosphate buffer at 37°C in pH 7.4, p=1.0 with NaCl

Esters	Methyl	Butyl	Pentyl	Cyclohexyl	Benzyl
$t_{1/2}$ (hr) 37°C pH 7.4	6.9	29.4	23.9	54.6	7.3

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To determine the effect of pH and buffer concentration on the stability of the ester, the hydrolysis of the butyl ester was studied in 0.05M, 0.20 M and 0.50 M phosphate buffer at 37°C and 20°C in pH 3.5, 5.5 and 7.4.

Tables 4 and 5 summarize the results of the degradation studies of L-dopa butyl ester in 0.05M, 0.20M and 0.50M phosphate buffer at pH 3.5, 5.5 and 7.4 at 20°C and 37°C.

Table 4: Half-lives for the degradation of L-dopa butyl ester in 0.05M, 0.20M, 0.50M phosphate buffer at 37°C at three different pHs (at 90% confidence intervals)

Butyl ester	$t_{1/2}$ (hr) at 37°C (0.05M buffer)	$t_{1/2}$ (hr) at 37°C (0.20M buffer)	$t_{1/2}$ (hr) at 37°C (0.50M buffer)
pH = 7.4	29 ± 2	18 ± 2	13 ± 1
pH = 5.5	339 ± 22	180 ± 10	121 ± 8
pH = 3.5	2573 ± 648	2199 ± 255	1159 ± 129

Table 5: Half-lives for the degradation of L-dopa butyl ester in 0.05m, 0.20M, 0.50M phosphate buffer at 20°C at three different pHs (at 90% confidence intervals)

Butyl ester	$t_{1/2}$ (hr) at 20°C (0.05M buffer)	$t_{1/2}$ (hr) at 20°C (0.20M buffer)	$t_{1/2}$ (hr) at 20°C (0.50M buffer)
pH = 7.4	197 ± 7	129 ± 4	74 ± 4
pH = 5.5	2038 ± 485	1050 ± 113	714 ± 182
pH = 3.5	8420 ± 5435	3785 ± 1658	2537 ± 1226

The above results show that the hydrolysis of these esters is subject to specific as well as general base catalysis.

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EXAMPLE 3: *In-vitro* Enzymatic Hydrolysis Studies*Analytical Procedures*

The HPLC system for in-vitro enzymatic studies also included: Applied Biosystems Solvent Delivery System 400, Fluorescence Detector 980; 5 ABI Analytical Kratos Division Spectroflow Static Mixer/Injector model 491; SpectraPhysics DataJet Integrator; Shimadzu Auto-Injector SIL-6A, Whatman Partisil 5 SCX column (4.6 mm x 100 mm), Whatman CO:PEL ODS Guard column (2 mm x 70 mm).

10 The mobile phase consisted of 0.05M phosphate buffer at pH 2.6, and acetonitrile, containing ethylenediaminetetraacetic acid disodium salt dehydrate 20 mg/l. The acetonitrile portion was adjusted according to the ester (see below). The flow rate was set at 1.0 ml/min. The excitation wavelength was set at 282 nm and the emission wavelength was set at 310 nm.

Rat Plasma

15 Five 200 μ l aliquot parts of rat plasma were added to five 100 μ l of a 0.05M, pH 6.0 phosphate buffer solution containing 1 mg/ml of each ester and the samples incubated at 37°C. The reactions were quenched at various times by adding 200 μ l of acetonitrile. The samples were centrifuged for 2 minutes. The supernatant was filtered through a 0.45 μ m filter and injected directly onto the 20 HPLC.

The rate of hydrolysis of each ester was determined from the slope of the linear plot of the logarithm of the residual ester concentration against time.

Rat Brain Homogenate

25 One part of whole rat brain tissue was homogenized with 5 parts of saline using a tissue grinder. Five 200 μ l aliquot parts of brain homogenate were added to five 100 μ l of a 0.05M, pH 6.0 phosphate buffer solution containing 1 mg/ml of the appropriate ester and incubated at 37°C. The reactions were quenched at

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various times by adding 200 μ l of acetonitrile. The samples were centrifuged for 2 minutes. The supernatant was filtered through a 0.45 μ m filter and injected directly onto the HPLC.

5 The rate of hydrolysis of each ester was determined from the slope of the linear plot of the logarithm of the residual ester concentration against time.

Rat Cerebrospinal Fluid

Five 50 μ l aliquot parts of rat CSF were added to five 50 μ l of a 0.05M, pH 6.0 phosphate buffer solution containing 1 mg/ml of the butyl ester and the samples incubated at 37°C. The reactions were quenched at various times by
10 adding 200 μ l of acetonitrile. The samples were centrifuged for 2 minutes. The supernatant was filtered through a 0.45 μ m filter and injected directly into the HPLC.

The rate of hydrolysis of butyl ester was determined from the slope of the linear plot of the logarithm of the residual ester concentration against time.

15 *Rat Nasal Perfusate*

Nasal perfusate was obtained from the rat nasal cavity by circulating 3 ml of saline into one nostril and collecting the saline solution from the other nostril. Circulating time was 3 minutes. The hydrolysis study was performed immediately following perfusion. Five 200 μ l aliquot parts of rat nasal perfusate
20 were added to five 100 μ l of a 0.05M, pH 6.0 phosphate buffer solution containing 1 mg/ml of the butyl ester and the samples incubated at 37°C. The reactions were quenched at various times by adding 200 μ l of acetonitrile. The samples were centrifuged for 2 minutes. The supernatant was filtered through a 0.45 μ m filter and injected directly into the HPLC.

25 The rate of hydrolysis of butyl ester was determined from the slope of the linear plot of the logarithm of the residual ester concentration against time.

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Results

In order to verify whether these prodrugs can generate L-dopa enzymatically in rat biological fluids, their rates of hydrolysis were determined in plasma, brain homogenate, and CSF fluid. Figure 15 shows that the hydrolysis of the butyl ester prodrug in rat plasma followed first-order kinetics and that the rate of generation of L-dopa is very rapid. Rapid generation of L-dopa was also observed in brain homogenate (Figure 16). The generation of L-dopa in CSF fluid (Figure 17), however, was much slower ($t_{1/2}$ = 34 minutes). The butyl ester was relatively stable in rat nasal perfusate. The half-life was greater than 2 hours, as shown in Figure 18. Since the nasal absorption of the prodrug is very rapid, a negligible amount will hydrolyze before it is absorbed into the systemic circulation.

Table 6 summarizes the half lives of several esters of L-dopa in rat plasma and rat brain homogenate. The half lives for the hydrolysis of the butyl ester in rat CSF and nasal perfusate are also reported in Table 6.

Table 6: Half-lives for the degradation of esters in rat plasma, rat brain homogenate, rat CSF and rat nasal perfusate at 37°C

Compound	$t_{1/2}$ (min) rat plasma	$t_{1/2}$ (min) rat brain	$t_{1/2}$ (min) rat CSF	$t_{1/2}$ (min) nasal perfusate
Methyl ester	0.82	0.96	---	---
Butyl ester	0.63	0.76	33.0	144.0
Pentyl ester	1.25	1.56	---	---
Cyclohexyl ester	14.1	10.1	---	---
Benzyl ester	0.36	0.96	---	---

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EXAMPLE 4: *In-vivo* Studies

The nasal absorption of L-dopa, L-dopa prodrugs, and dopamine was studied using an in-vivo experimental technique described by Hussain et al.^{33,37} (Figure 12).

5 ***Analytical Procedures for the in vivo Studies***

The HPLC system for *in vivo* studies included: Applied Biosystems Solvent Delivery System 400, Applied Biosystems 429A Integrator, ABI Analytical Kratos Division Spectroflow Static mixer/Injector model 591; BAS Amperometric Detector LC-4B (operated at +0.8 V vs. a Ag/AgCl reference electrode), TOSOH TSK-GEL ODS-80Tm column (4.6 mm x 150 mm),
10 Whatman CO:PEL ODS Guard column (2 mm x 70 mm).

L-dopa and dopamine were measured in plasma, brain, and cerebrospinal fluid (CSF) by a previous reported high performance liquid chromatographic (HPLC) procedure using an electrochemical detector³⁶, with a slight modification.
15 The mobile phase consisted of 0.05M phosphate buffer at pH 2.9, heptane sulfonate sodium salt 500 mg/l, and ethylenediaminetetraacetic acid disodium salt dehydrate 15 mg/l. The flow rate was set at 1.5 ml/min. The retention times were 13 minutes for L-dopa, 17 minutes for dihydroxyphenylamine(internal standard) and 29 minutes for dopamine.

20 Male Sprague-Dawley rats weighing 250-275 gm were used. Animals were fasted overnight before the experiment, but water was given *ad libitum*. Reserpine (4 mg/kg) was given 24 hours prior to the experiment. All surgical procedures were performed under anesthesia; i.e., intraperitoneal injection of pentobarbital (40 mg/kg). An incision was made in the neck, and the trachea
25 was cannulated with a polyethylene tube. A closed tube was inserted through the esophagus to the posterior part of the nasal cavity. The nasopalatine passage was closed with an adhesive agent to prevent drainage of the drug from the nasal cavity to the mouth.

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Blood samples were collected from a cannula inserted into the femoral artery. For intravenous administration, the jugular vein was cannulated for administering the dose.

To determine the residual amount of dopamine in the nasal cavity, the
5 cavity was washed with 2 ml of 0.05M, pH 6.0 phosphate buffer. The dopamine concentration was determined by HPLC.

Preparation of the Solutions

Ester prodrugs solutions at 4, 20, and 40 mg/kg/0.2 ml equimolar doses of L-dopa were freshly prepared by using 0.05M phosphate buffer at pH 6.0.

10 Solutions of L-dopa were prepared by first dissolving the compound in 1N hydrochloric acid then using 0.5M phosphate buffer at pH 7.4 to adjust the solution to pH 4.

Solutions for dopamine were prepared at 20mg/kg/0.2ml by using 0.05M phosphate buffer at pH 6.0.

15 For nasal administration, aqueous solutions of L-dopa or equimolar prodrugs were administered through the nostril using a microsyringe. For intravenous administration, the same dose of the drug was injected through the jugular vein.

Sample Collection after Nasal and Intravenous Administrations

20 For intravenous administration studies, blood samples were collected at 0, 2, 5, 10, 15, 30, 45, 60, 90, and 120 minutes. For nasal administration studies, blood samples were collected at 0, 5, 10, 15, 20, 30, 40, 60, 90, and 120 minutes. After immediate centrifugation (3000 xg for 3 min), the plasma was separated. The animal was sacrificed after the last sample was obtained and the
25 brain was carefully removed. The olfactory bulb and striatum were carefully separated from the brain. Purification of the biological samples for L-dopa and dopamine analysis was carried out using a modified alumina adsorption

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procedure (described in Section 4.1) and the samples were then analyzed by HPLC.

Extraction of L-dopa

Plasma samples were mixed with 5 μ l of 2% Na₂EDTA and 5 μ l of 5% sodium metabisulfite in normal saline. The samples were kept frozen until extraction. L-dopa was isolated by a modification of the alumina adsorption procedure of A.H. Anton³⁴. (Alumina activation was mentioned in Section 4.1) Each plasma sample (50 μ l) was mixed with 70 mg of activated aluminum gel, 0.2 ml of 2M Tris buffer (pH 8.6), 0.1 ml of 2N NaOH, and 10 μ l of 3,4-dihydroxybenzylamine aqueous solution as an internal standard in a glass test-tube for 30 min. After mixing, the alumina was washed once with 8 ml of 10mM Tris buffer (pH 8.6) and twice with 8 ml of distilled water adjusted to pH 7.0 with 0.1N NaOH. After the water was aspirated, L-dopa was eluted with 0.3 ml of 0.8N HCl. The samples obtained were frozen until HPLC analysis.

15 Results

Studies with L-dopa and its Prodrugs

Since the L-dopa esters are converted to L-dopa very rapidly in rat plasma, analysis of L-dopa in the plasma following the nasal administration of the prodrugs should accurately reflect the absorption profiles of these esters. Figure 19 shows plasma L-dopa levels after nasal administration of L-dopa and the prodrugs at a dose of 4 mg/kg L-dopa equivalent.

Such rapid and complete absorption was also observed at higher administered doses. This is shown in Figure 20 for three different doses of the butyl ester.

25 The area under the curve for 4 and 20 mg/kg doses was calculated by using the STRIPTM computer program. The area under the plasma-time curves were truncated at 120 minutes. The AUCs were 92.283, and 521.55 (μ g/ml*min) for 4 mg/kg and 20 mg/kg doses, respectively. The AUCs are

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proportional to the administered doses. The rate of absorption of the butyl ester and the rate of elimination of L-dopa were calculated using the data in Figure 20 and were found to be 0.128 min^{-1} and 0.011 min^{-1} , respectively.

The bioavailability of the butyl ester following nasal administration was also obtained by comparing the AUCs after intravenous and nasal administrations. Figure 21 shows the plasma level profiles following the nasal and intravenous administrations of the butyl ester at the 20 mg/kg L-dopa equivalent dose. The AUCs were calculated to be $584.29 \mu\text{g/ml}\cdot\text{min}$ for the intravenous route and $521.55 \mu\text{g/ml}\cdot\text{min}$ for the nasal route. The nasal bioavailability is about 89.3% of that of the intravenous administration.

Studies with Dopamine

In order to obtain a clear picture of the pharmacokinetic profile of the butyl ester following its nasal administration to rats (Figure 22), the absorption and elimination of dopamine in the same animal model was examined.

Dopamine was found to be rapidly eliminated following intravenous administration as shown in Figure 23. The elimination rate constant was found to be 10 times faster than that of L-dopa and was estimated to be 0.118 min^{-1} .

The nasal absorption of dopamine was found to be relatively slow and incomplete as shown in Figure 23. At doses of 20 mg/kg, the absorption phase was long and at the end of experiment, about 68% of the administered dose was recovered from the nasal cavity. Such a slow rate of absorption could not be attributed to the partition coefficient, since the partition coefficient of L-dopa in the same solvent system is similar to that of dopamine.

It may be possible that dopamine retards its own absorption due to its vasoconstrictive effect. Previous studies with phenylephrine showed that the compound inhibited the absorption of aspirin from the nasal cavity. It would appear from the magnitude of the rate constants of L-dopa and dopamine metabolism that the plasma level of dopamine after nasal administration of the prodrug would be too small to be detected.

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CSF and Olfactory Bulb L-dopa Levels following Nasal administration of the Prodrugs

The cerebrospinal fluid and olfactory bulb concentrations of L-dopa following the intravenous and nasal administration of the butyl ester at 20 mg/kg L-dopa equivalent dose are shown in Figure 24 and Figure 25. It is evident that the cerebrospinal fluid and the olfactory bulb have higher concentrations of L-dopa following nasal administration than following intravenous administration. These data suggest that the butyl ester can reach the CSF or olfactory bulb via a direct pathway.

Dependency of the CSF and Olfactory Bulb L-dopa Levels on the Lipophilicity of the Prodrugs

It has been shown previously⁸ that the concentration in the CSF of a series of sulfa drugs administered nasally is related to the lipophilicity of the compounds. To reconfirm the above observation, two prodrugs of L-dopa, i.e. butyl and methyl esters, with different partition coefficients were chosen for this study. The results are shown in Table 7.

Table 7: Relationship of the partition coefficients and L-dopa levels in the plasma, CSF and olfactory bulb following nasal administration

Esters	P.C. ^a	L-dopa Concentration (µg/ml)					
		Plasma		CSF		Olfactory Bulb	
		60 min	120 min	60 min	120 min	60 min	120 min
Methyl	0.252	2.360 ±0.200	2.915 ±0.120	3.250 ±0.560	2.540 ±0.012	87.20 ±20.57	87.90 ±25.88
Butyl	7.166	4.273 ±2.31	2.234 ±0.493	13.92 ±3.655	5.692 ±4.835	250.2 ±182.11	98.40 ±33.78

^aPartition coefficient measured at 20°C, octanol/pH 7.4 0.05M phosphate buffer; n=2 for Methyl Ester, n=3 for Butyl Ester

The data in Table 7 shows that the more lipophilic drug, i.e. the butyl ester, afforded higher L-dopa levels in both the CSF and the olfactory bulb than

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the methyl ester. For both esters, the olfactory bulb L-dopa levels are higher than CSF L-dopa levels.

Plasma Levels of Dopamine following the Nasal Administration of L-dopa Butyl Ester

5 Although L-dopa plasma levels were high following the nasal administration of 20 mg/kg L-dopa equivalent of the butyl ester, dopamine plasma levels were very low. Using the kinetic model shown in Figure 26 and the analytical solutions in Example 6, plasma L-dopa and dopamine levels were generated by using a BASIC computer program. The results are shown in Table 10 8 and Figure 27. It would appear from the above that the nasal administration of the butyl ester of L-dopa does not contribute significantly to dopamine plasma levels.

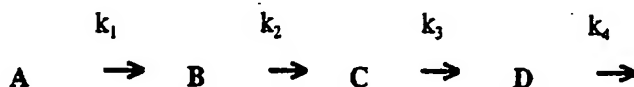
Table 8: Experimental and calculated plasma levels($\mu\text{g/ml}$) for L-dopa and dopamine in the rat

15	Time	L-dopa (experiment)	L-dopa (calculated)	dopamine (experiment)	dopamine (calculated)
	control	0.000		0.096	
	5	5.477	3.128	0.089	7.41×10^{-8}
	10	6.314	5.135	0.155	2.39×10^{-7}
	15	6.441	6.013	0.170	3.90×10^{-7}
20	20	6.763	6.301	0.097	4.98×10^{-7}
	40	5.436	5.623	0.117	5.94×10^{-7}
	60	4.273	4.564	0.100	5.08×10^{-7}
	90	3.268	3.295	0.109	3.70×10^{-7}
	120	2.234	2.376	0.070	2.69×10^{-7}

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EXAMPLE 5: Mathematical Modeling

Figure 44 can be simplified to the following rate equations:



- 5 A = Concentration of the butyl ester of L-dopa in rat nasal cavity
 B = Concentration of the butyl ester of L-dopa in rat plasma
 C = concentration of L-dopa in rat plasma
 D = Concentration of dopamine in rat plasma
 10 k_1 = Absorption rate constant for the butyl ester of L-dopa from the rat nasal cavity
 k_2 = Hydrolysis rate constant from butyl ester to L-dopa
 k_3 = Metabolism rate constant from L-dopa to dopamine
 k_4 = Metabolism rate constant for dopamine

- 15 The rates of concentration change for each of the components are as follows:

$$\frac{dA}{dt} = -k_1 A \quad (1)$$

$$\frac{dB}{dt} = k_1 A - k_2 B \quad (2)$$

$$\frac{dC}{dt} = k_2 B - k_3 C \quad (3)$$

$$\frac{dD}{dt} = k_3 C - k_4 D \quad (4)$$

Differential equations 9 to 12 can be solved using Laplace Transforms.
 The analytic solutions of equations 9 to 12 are as follows:

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$$A = A_0 e^{(-k_1 t)} \quad (5)$$

$$\frac{-k_1 A_0}{k_1 - k_2} [e^{-k_1 t} - e^{-k_2 t}] \quad (6)$$

$$\frac{k_1 k_2 A_0}{(k_1 - k_2)(k_1 - k_3)(k_2 - k_3)} \times [(k_2 - k_3)e^{(-k_1 t)} - (k_1 - k_3)e^{(-k_2 t)} - (k_1 - k_2)e^{(-k_3 t)}] \quad (7)$$

$$\frac{k_1 k_2 k_3 A_0}{(k_1 - k_2)(k_1 - k_3)(k_1 - k_4)(k_2 - k_3)(k_2 - k_4)(k_3 - k_4)} \times [-(k_2 - k_3)(k_2 - k_4)(k_3 - k_4)e^{(-k_1 t)} + (k_1 - k_3)(k_1 - k_4)(k_3 - k_4)e^{(-k_2 t)} - (k_1 - k_2)(k_1 - k_4)(k_2 - k_4)e^{(-k_3 t)} + (k_1 - k_2)(k_2 - k_3)(k_1 - k_3)e^{(-k_4 t)}] \quad (8)$$

From the above analytic solutions, a computer program in BASIC was written to calculate A, B, C, and D as a function of time. The following data were used to calculate A, B, C, and D: $A_0 = 6000 \mu\text{g}$, the initial dose; $k_1 = 0.1277 \text{ min}^{-1}$, rate of absorption; $k_2 = 1.1 \text{ min}^{-1}$, rate of hydrolysis; $k_3 = 0.0109 \text{ min}^{-1}$, rate of elimination of L-dopa; $k_4 = 0.1177 \text{ min}^{-1}$, rate of elimination of dopamine.

REFERENCES

1. Jankovic et al. *Parkinson's Disease and Movement Disorders* (2nd ed.) Williams & Wilkins, 1993.
2. Thibodeau et al. *Anatomy & Physiology* (2nd ed.) Mosby-Year Book, Inc., 1993.
3. Nutt et al. "Parkinson's Disease" Porter RJ, ed. *100 Maxims in Neurology*; vol 2, 1992.
4. Gilman et al. *The Pharmacological Basis of Therapeutics*; (8th ed.) 1990
5. Hussain et al. "Nasal Absorption of Propranolol from Different Dosage Forms by Rats and Dogs" *J. Pharmaceut. Sci.* 69: 1411-1413, 1980.
- 10 6. Hussain et al. "Nasal Absorption of Propranolol in Humans" *J. Pharmaceut. Sci.*; 69: 1240, 1980.
7. Hussain et al. "Nasal Absorption of Propranolol in Rats" *J. Pharmaceut. Sci.* 68:1196, 1979.
- 15 8. Sakane et al.; "The Transport of a Drug to the Cerebrospinal Fluid Directly from the Nasal Cavity: the Relation to the Lipophilicity of the Drug" *Chem. Pharm. Bull.* 39: 2456-2458, 1991.
9. Gomez et al. "Levodopa" In: Flory K, ed. *Analytical Profiles of Drug Substances*; Academic Press, Inc., 189-223, vol 5, 1976
- 20 10. Dempsey et al. "Pharmaceutical Design and Development of a Sinemet Controlled-Release Formulation" *Neurology* 39:2024, 1989.
11. Sasahara et al. "Dosage Form Design for improvement of Bioavailability of Levodopa V: Absorption and Metabolism of levodopa in intestinal Segments of Dogs" *J. Pharmaceut. Sci.* 70:1157-1160, 1981.
- 25 12. Sasahara et al. "Dosage Form Design for Improvement of Bioavailability of Levodopa IV: Possible Causes of Low Bioavailability of Oral Levodopa in Dogs." *J. Pharmaceut. Sci.* 70:730-733, 1981.
13. Wingard et al. *Human Pharmacology*. Mosby Year Book, 1991

14. Sasahara et al. "Dosage Form Design for improvement of Bioavailability of Levodopa III: Influence of Dose on Pharmacokinetic Behavior of Levodopa in Dogs and Parkinsonian Patients." *J. Pharmaceut. Sci.* **69**: 1374-1378, 1980.
- 5 15. Cedarbaum "Anti-Parkinsonian Drugs." *Clin. Pharmacokinet.* **13**: 141-178, 1987.
16. Nutt "The Effect of Carbidopa on the Pharmacokinetics of Intravenously Administered Levodopa: the Mechanism of Action in the Treatment of Parkinsonism." *Ann. Neurol.* **18**:537-543, 1986.
- 10 17. Cedarbaum et al. "Effect of Supplemental Carbidopa on the Bioavailability of L-dopa." *Clin. Neuropharmacol.* **9**:153-159, 1986.
18. Quinn et al. "Control of On/Off Phenomenon by Continuous Intravenous Infusion of Levodopa." *Neurology* **34**:1131-1136, 1984.
19. Shoulson et al. "On-Off Response." *Neurology* **25**:1144-1148, 1975.
- 15 20. Bennett et al. "Continuous Oral Administration of L-Dihydroxy-phenylalanine (L-dopa) Solution to Patients with Advanced Parkinson's Disease." *Clin. Neuropharmacol.* **12**:285-292, 1989.
21. Nutt et al. "Levodopa Pharmacokinetics and Pharmacodynamics in Fluctuating Parkinsonian Patients." *Neurology* **36**: 739-744, 1986.
- 20 22. Hardie et al. "On-Off Fluctuations in Parkinson's Disease." *Brain* **107**: 487-506, 1984
23. Kurlan et al. "Duodenal and Gastric Delivery of Levodopa in Parkinsonism." *Ann. Neurol.* **23**:589-595, 1988.
24. Feldman et al., "Double-Blind Comparison of Standard Sinemet and Sinemet CR in Patients with Mild-to-Moderate Parkinson's Disease." *Neurology* **39**:96-101, 1989.
- 25 25. Hutton et al. Treatment of Chronic Parkinson's Disease with Controlled-Release carbidopa/levodopa." *Neurology* **45**:861-864, 1988.

26. Cedarbaum et al., "A Pharmacokinetic and Pharmacodynamic Comparison of Sinemet CR (501200) and Standard Sinemet (251100)." *Neurology* 39(suppl 2):38-44, 1989.
27. Cedarbaum JM, Breck L, Kutt H, McDowell FH; *Controlled-Release Levodopa/Carbidopa II: Sinemet CR4 Treatment of Response Fluctuations in Parkinson's Disease*; "Neurology; 37, 1607-1612, 1987
28. Bordor NS, Sloan KB, Hussain AA; *Novel, Transient Pro-Drug Forms of L-dopa*; "United States Patent; 3,891,696, 1975
29. Hanson LCF, Utley JD; *Biochemical and Behavioral Effects of L-dopa Methyl Ester in Cats Treated with Reserpine*; "Psychopharmacologia; 8, 140-144, 1965
30. Bordor N, Sloan KB, Higuchi T, Sasahara K; *Improved Delivery through Biological membranes. 4. Prodrugs of L-dopa*; "Journal of Medicinal Chemistry; 20(11), 1435-1445, 1977
31. Cooper et al. "L-dopa Esters as Potential Prodrugs: Behavioural Activity in Experimental Models of Parkinson's Disease." *J. Pharm. Pharmacol.* 39: 627-635, 1987.
32. Fix et al. "Short-Chain Alkyl Esters of L-dopa as Prodrugs for Rectal Absorption." *Pharmaceut. Res.*, 6:501-505, 1989.
33. Huang CH, Kimura R, Bawarshi-Nassar R, Hussain A; "Mechanism of Nasal Absorption of Drugs II: Absorption of L-Tyrosine and the Effect of Structural Modification on its Absorption." *J. Pharmaceut. Sci.* 74:1298-1301, 1985.
34. Anton et al., "A Study of the Factors Affecting the Aluminum Oxide-Trihydroxyindole Procedure for the Analysis of Catecholamines." *J. Pharmacol. Exp. Ther.* 138: 360-375, 1962.
35. Patel et al., "Synthesis of Benzyl Esters of α -Amino Acids." *J. Org. Chem.* 30: 3573, 1965.

-37-

36. Causon et al., "HPLC with Amperometric Detection of Plasma L-3,4-Dihydroxyphenylalanine in Parkinsonian Patients." *J. Chromatog*, 277: 115, 1983.
37. Huang et al., "Mechanism of Nasal Absorption of Drugs I:
5 Physicochemical Parameters Influencing the Rate of In Situ Nasal
Absorption of Drugs in Rats; *J. Pharmaceut. Sci.* ; 74: 608-611, 1985.

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What is claimed is:

1. A method for treating dopamine deficiency comprising intranasally administering to a patient in need of such treatment an effective amount of pharmaceutical composition comprising a water-soluble prodrug of L-dopa.
- 5 2. The method of claim 1, wherein the water soluble prodrug is an ester of L-dopa.
3. The method of claim 1, wherein the water-soluble prodrug is an alkyl, cycloalkyl, or aryl ester of L-dopa.
4. The method of claim 3, wherein the alkyl ester of L-dopa is selected from
10 the group consisting of methyl, butyl, and pentyl esters.
5. The method of claim 3, wherein the cycloalkyl ester of L-dopa is cyclohexyl ester.
6. The method of claim 3, wherein the aryl ester of L-dopa is benzyl ester.
7. The method of claim 1, which further comprises a pharmaceutically
15 acceptable carrier for the water-soluble prodrug of L-dopa.
8. The method of claim 7, wherein the carrier is aqueous.
9. The method of claim 7, wherein the carrier is 0.05 M phosphate buffered saline.

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10. A method for treating Parkinson's disease comprising intranasally administering to a patient in need of such treatment an effective amount of pharmaceutical composition comprising a water-soluble prodrug of L-dopa.
11. The method of claim 10, wherein the water soluble prodrug is an ester of
5 L-dopa.
12. The method of claim 10, wherein the water-soluble prodrug is an alkyl, cycloalkyl, or aryl ester of L-dopa.
13. The method of claim 12, wherein the alkyl ester of L-dopa is selected from the group consisting of methyl, butyl, and pentyl esters.
- 10 14. The method of claim 12, wherein the cycloalkyl ester of L-dopa is cyclohexyl ester.
15. The method of claim 12, wherein the aryl ester of L-dopa is benzyl ester.
16. The method of claim 10, which further comprises a pharmaceutically acceptable carrier for the water-soluble prodrug of L-dopa.
- 15 17. The method of claim 16, wherein the carrier is aqueous.
18. The method of claim 17, wherein the carrier is 0.05 M phosphate buffered saline.

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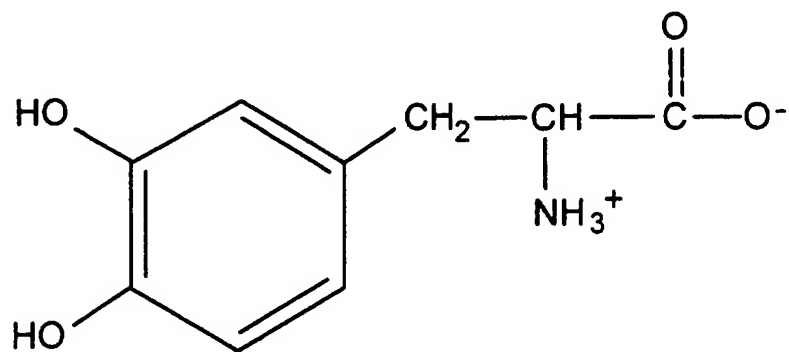


FIG. 1

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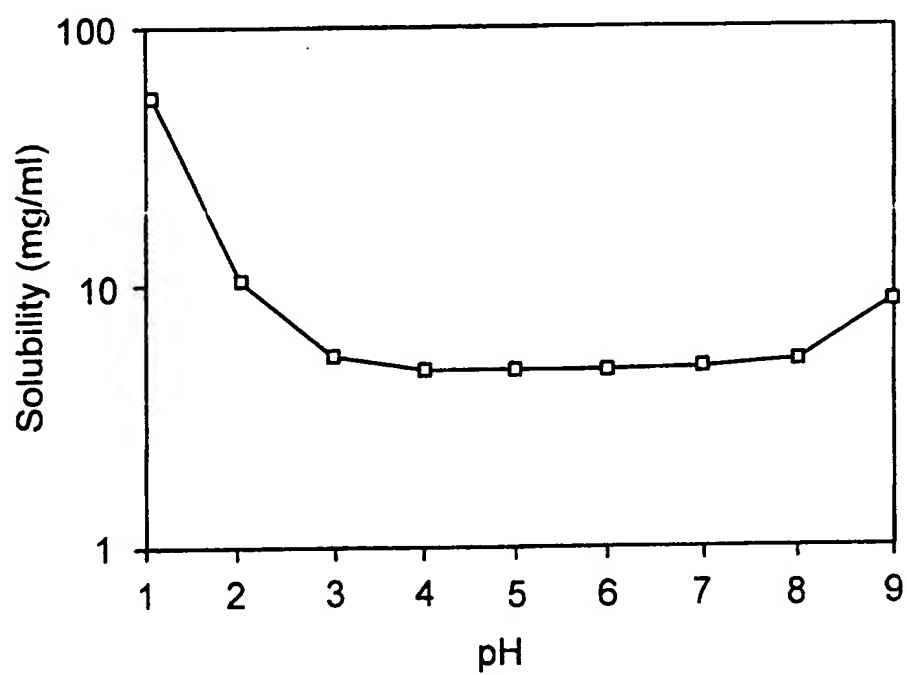


FIG. 2

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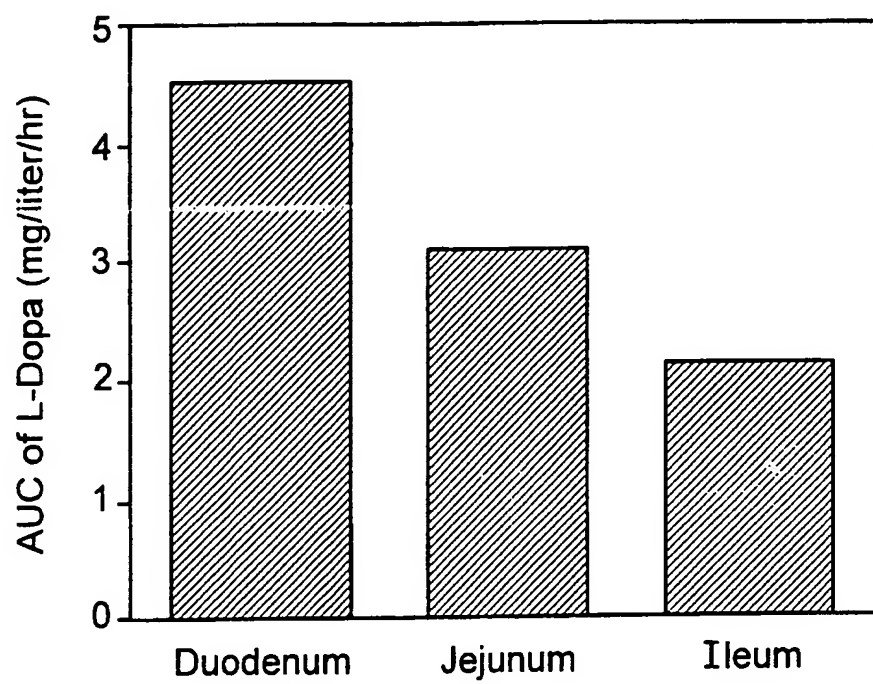


FIG. 3

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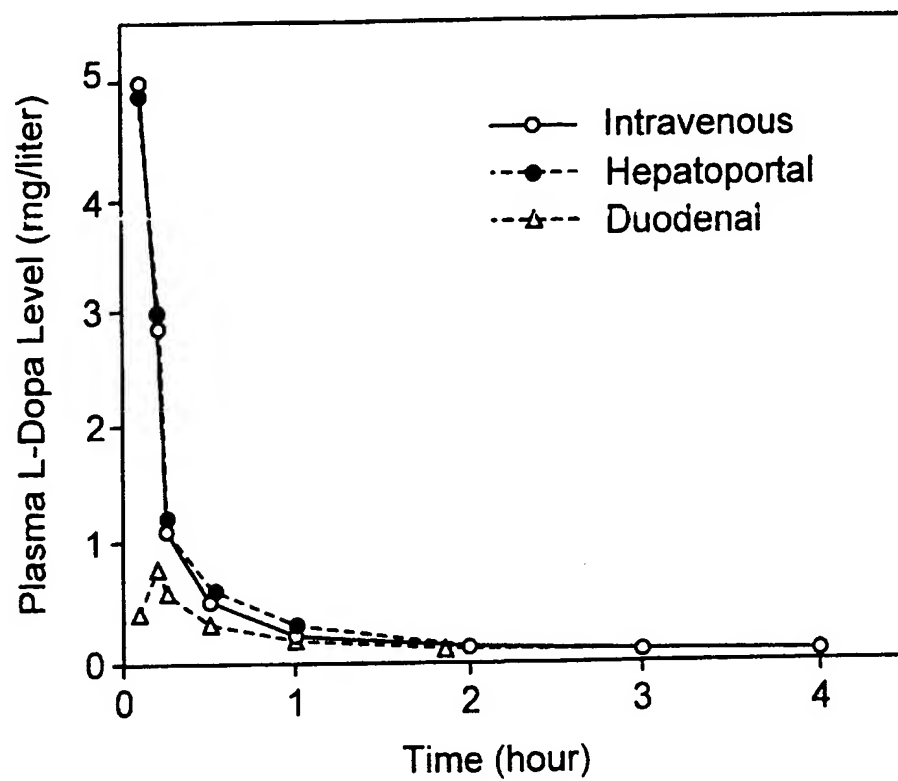


FIG. 4

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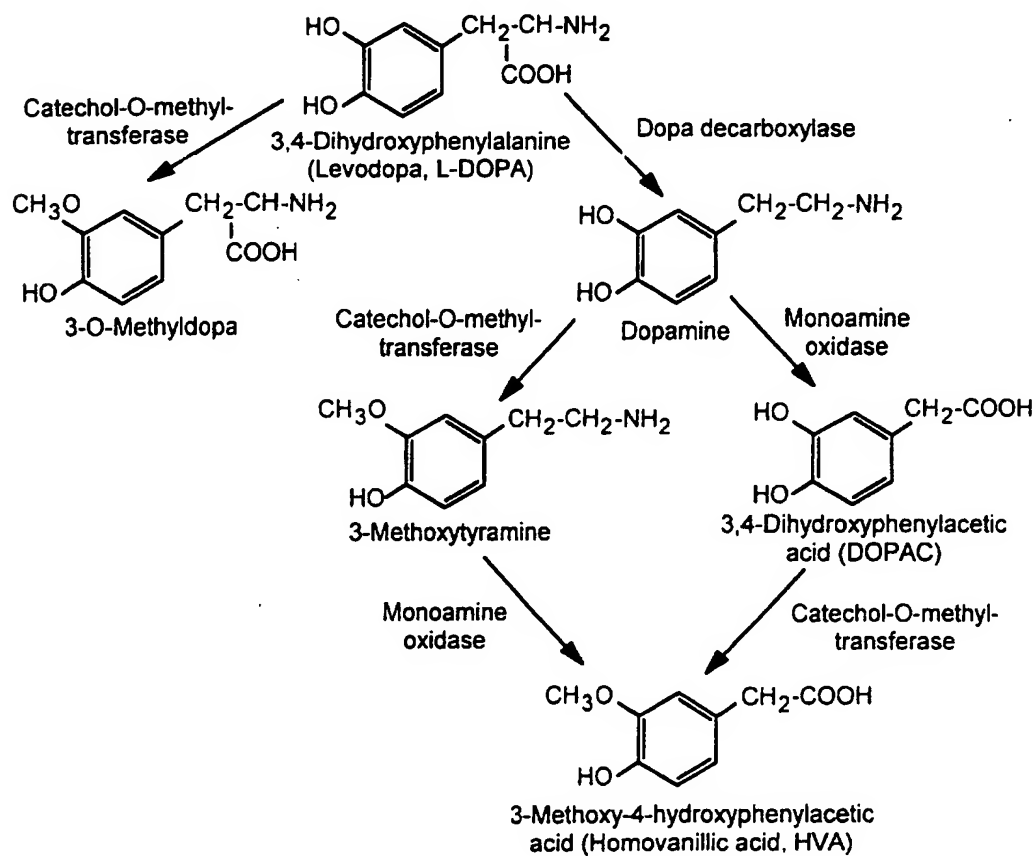


FIG. 5

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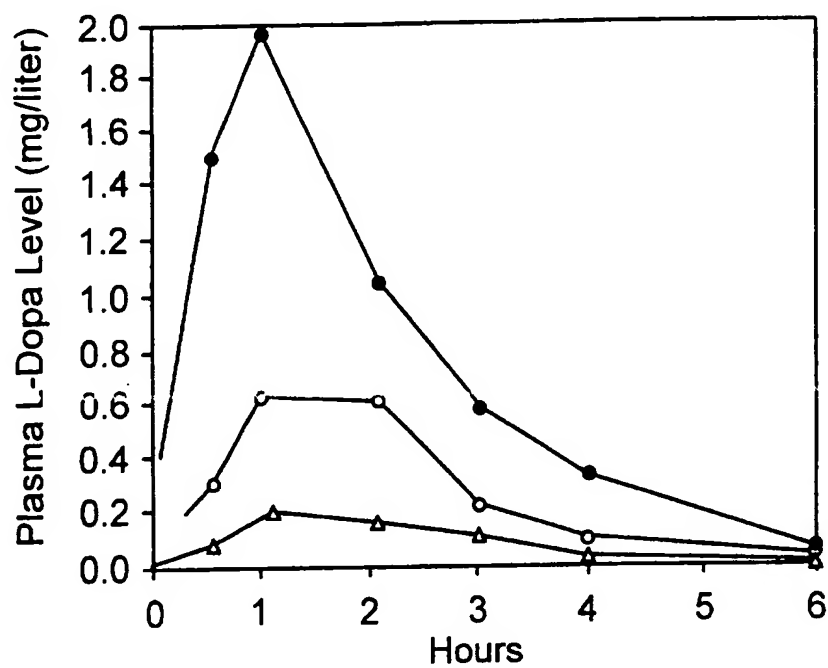


FIG. 6A

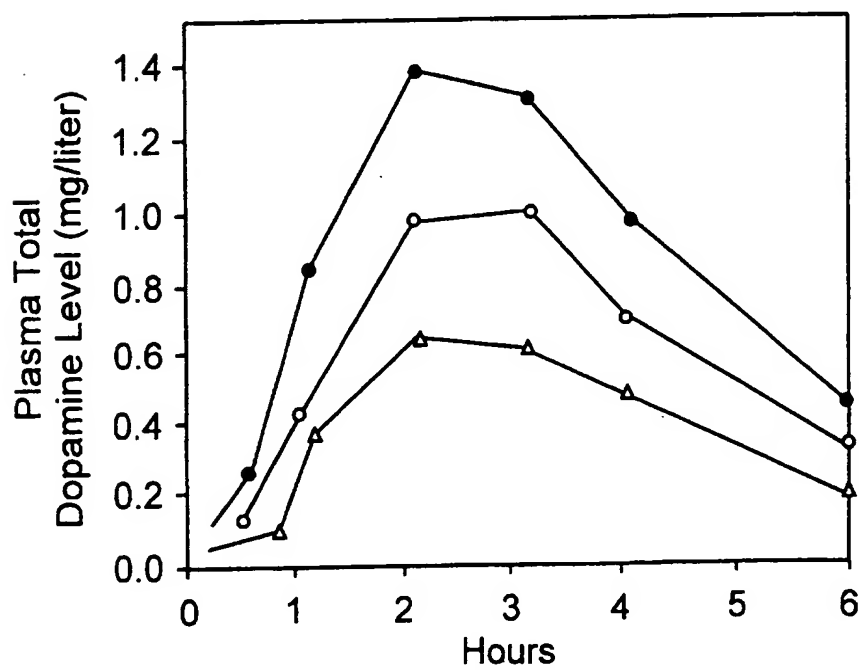


FIG. 6B

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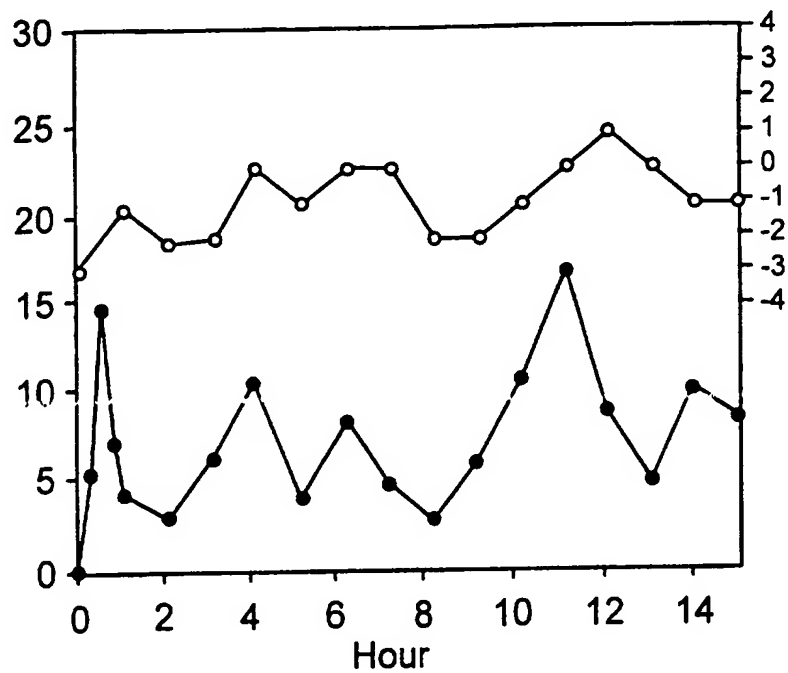


FIG. 7A

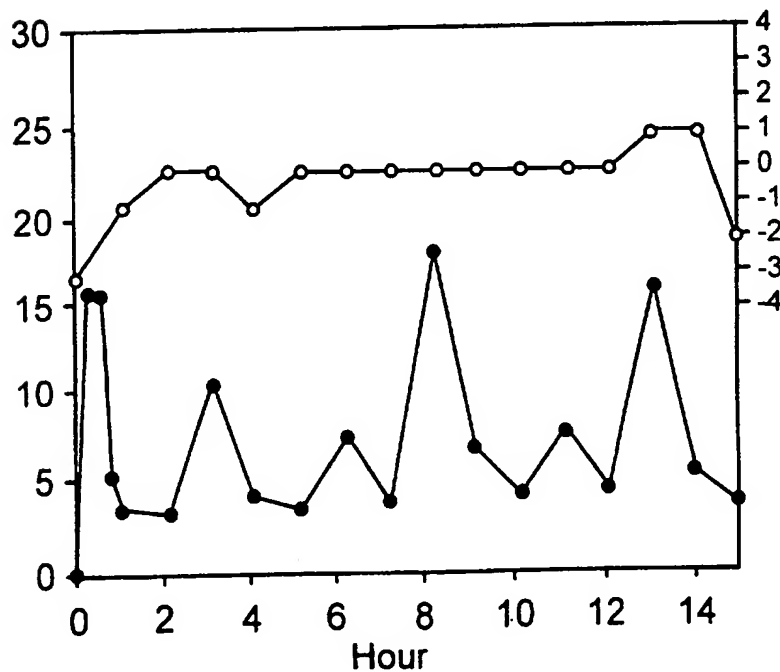


FIG. 7B

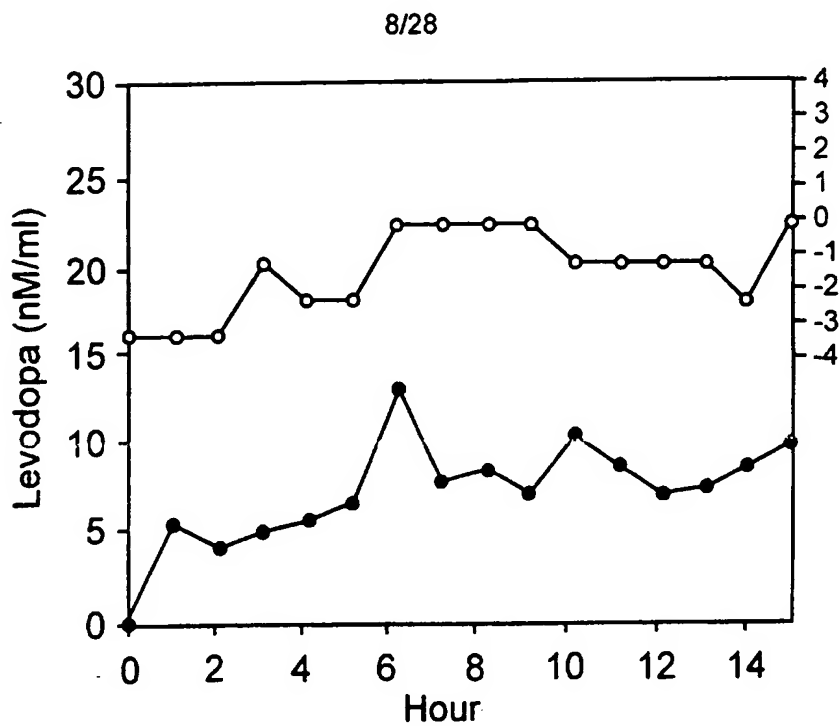


FIG. 7C

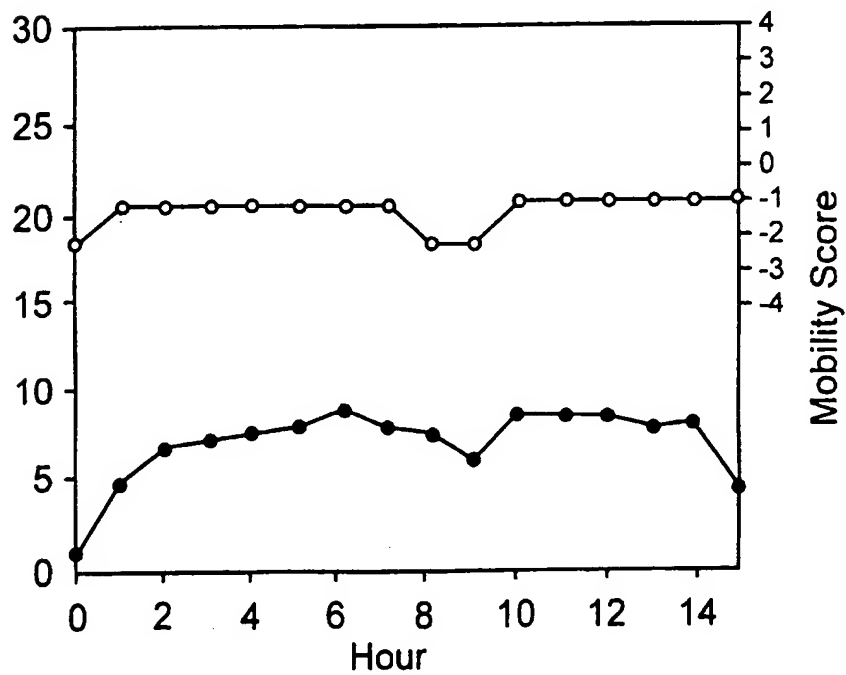


FIG. 7D

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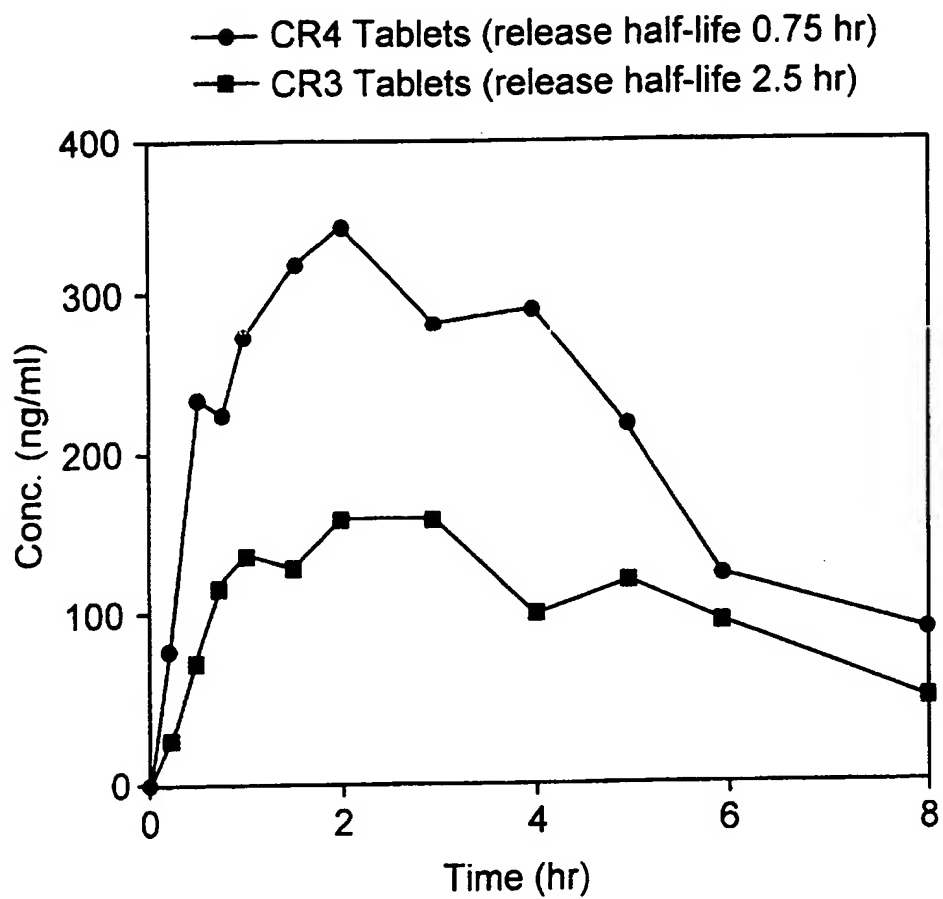


FIG. 8

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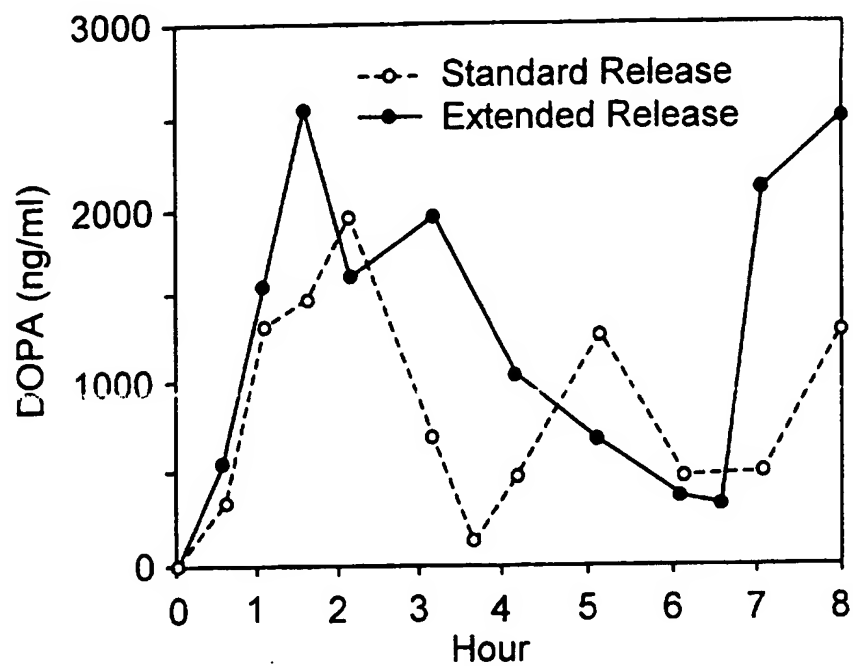


FIG. 9A

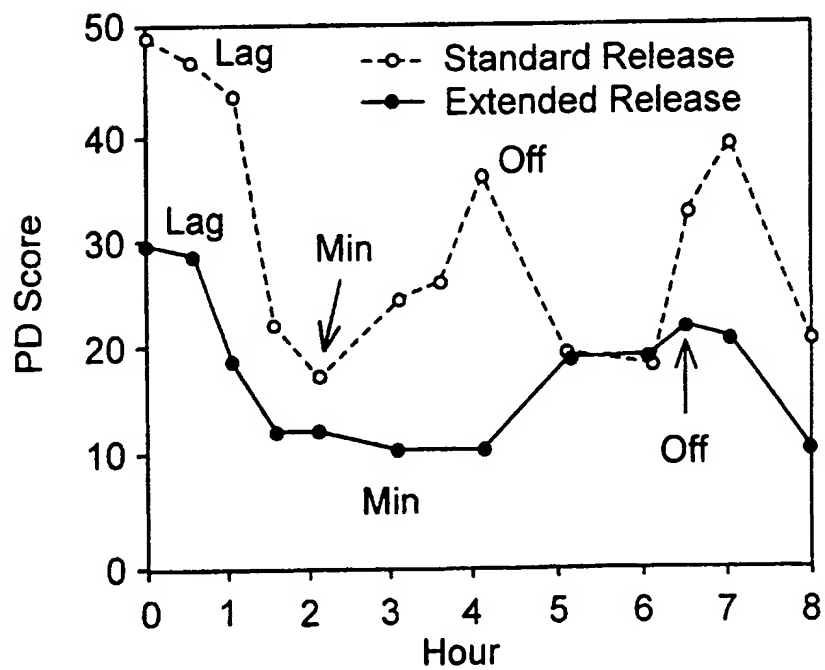


FIG. 9B

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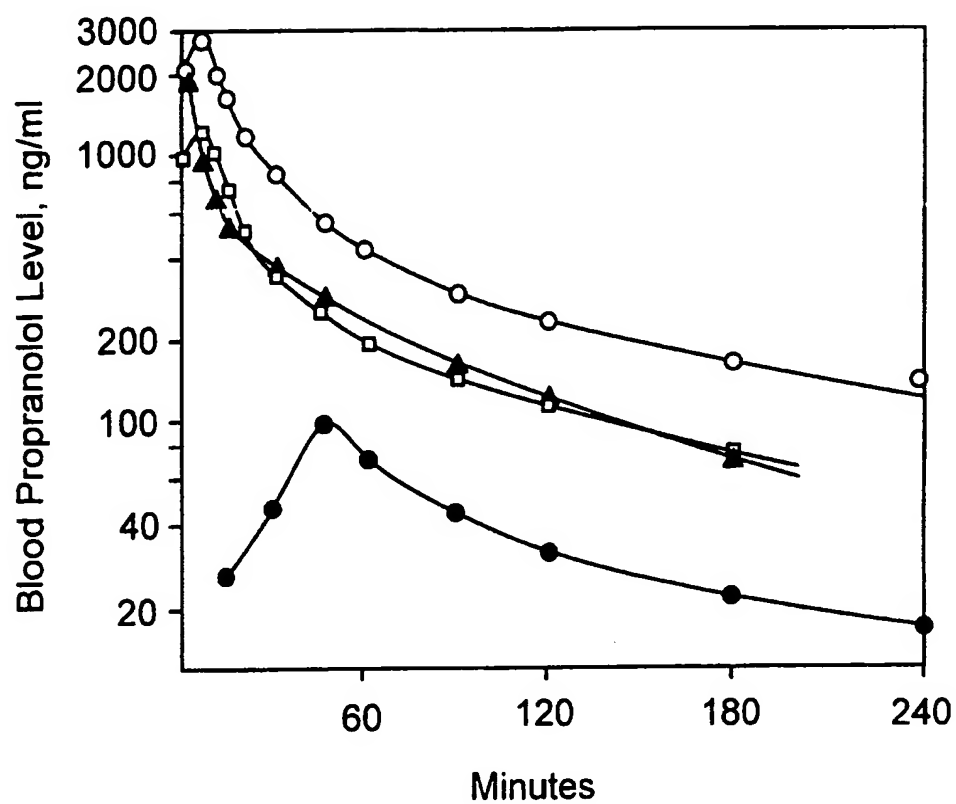


FIG. 10

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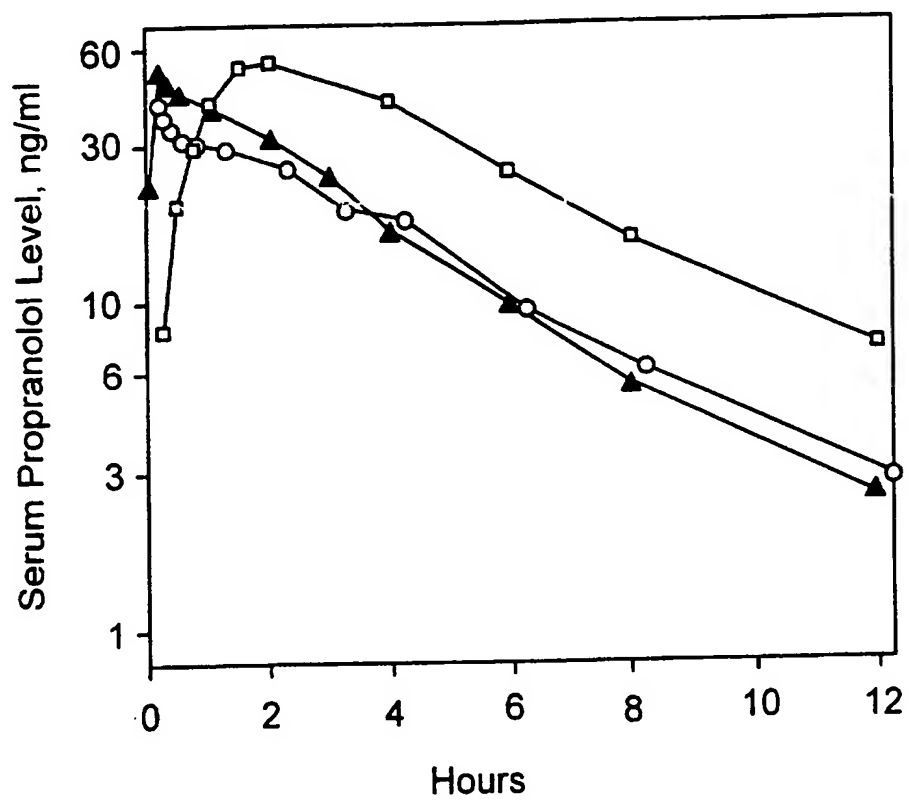


FIG. 11

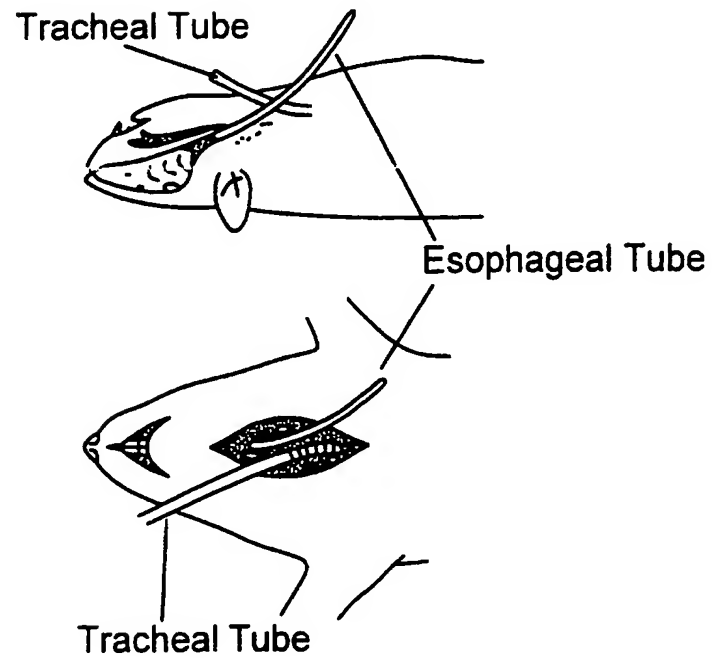


FIG. 12

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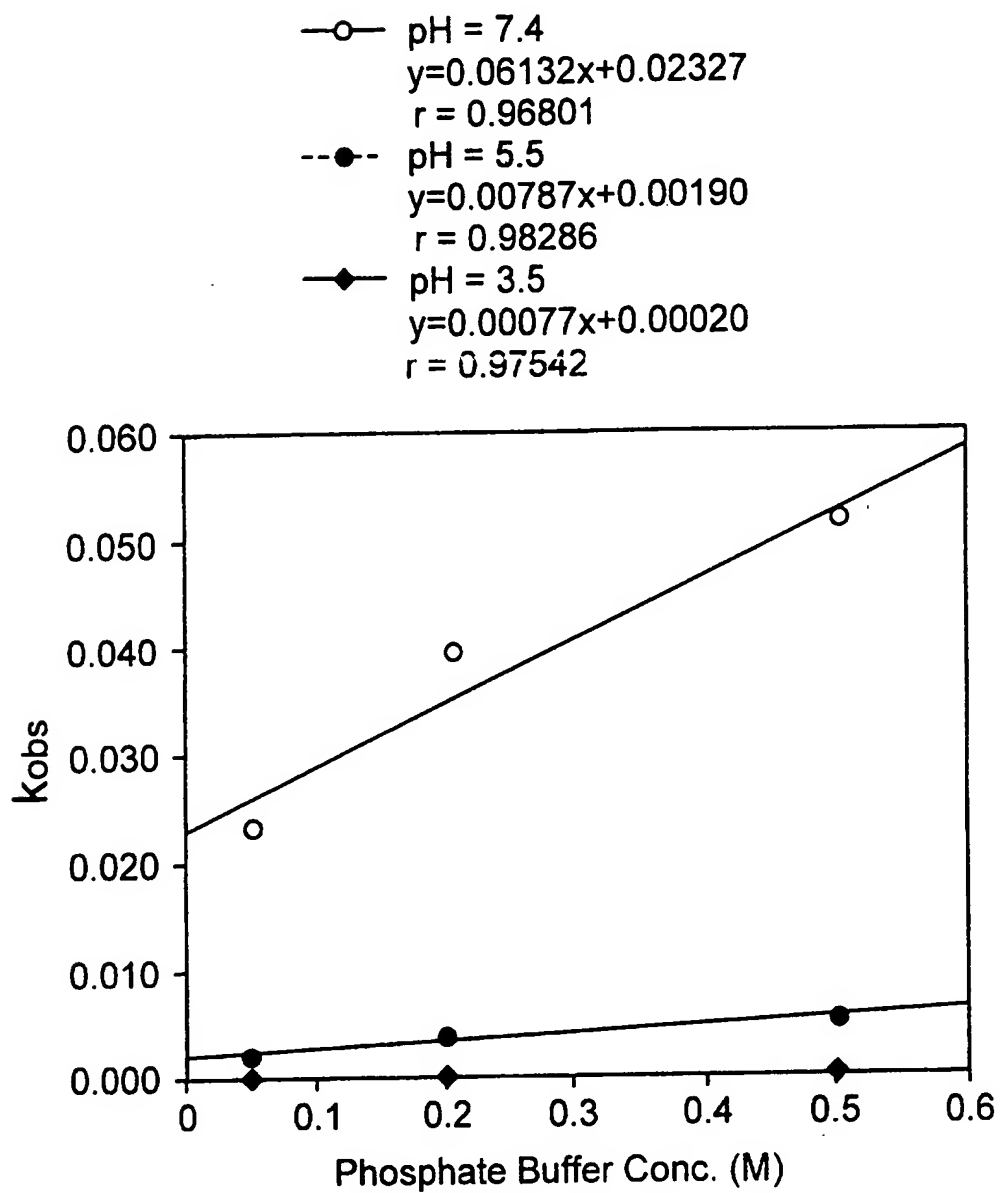


FIG. 13

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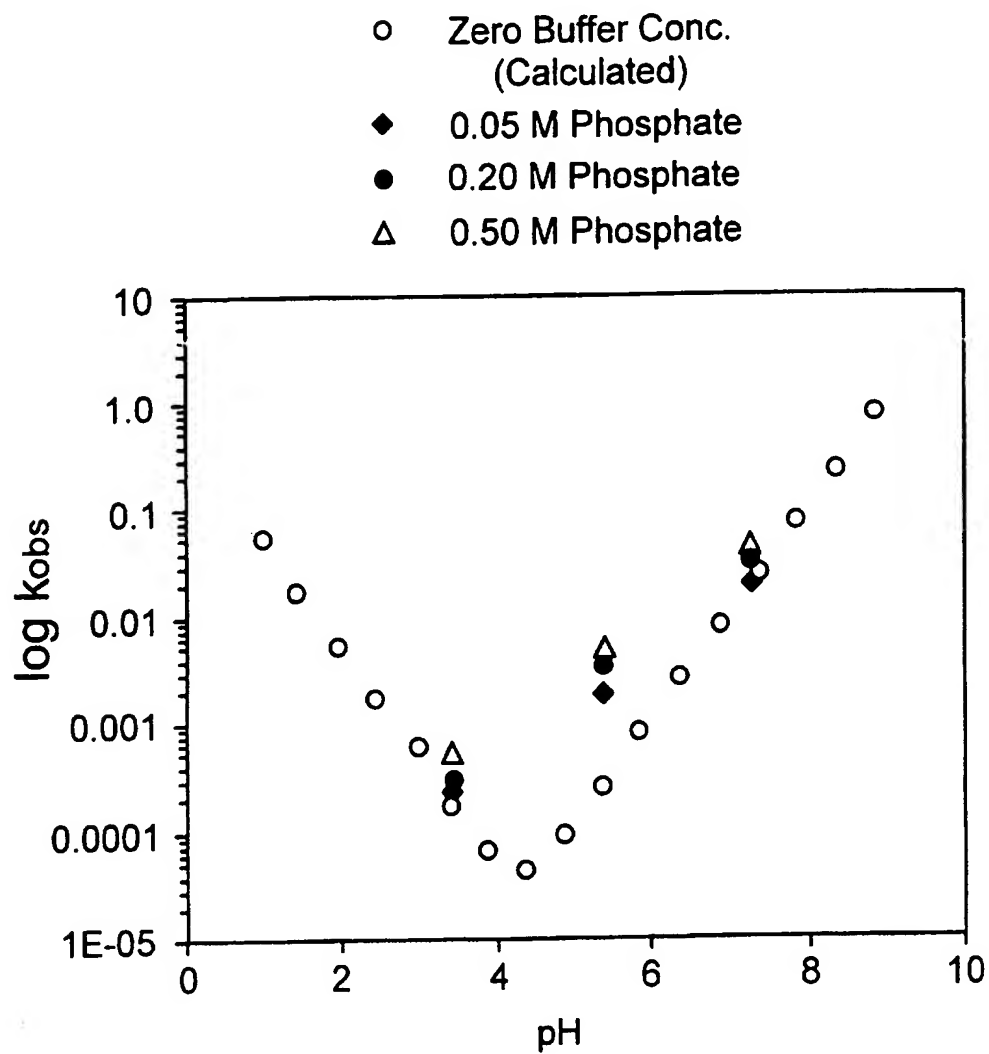


FIG. 14

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$$y = -1.014 \ln x + 11.881$$
$$r = 0.996, t_{1/2} = 0.63 \text{ min}$$

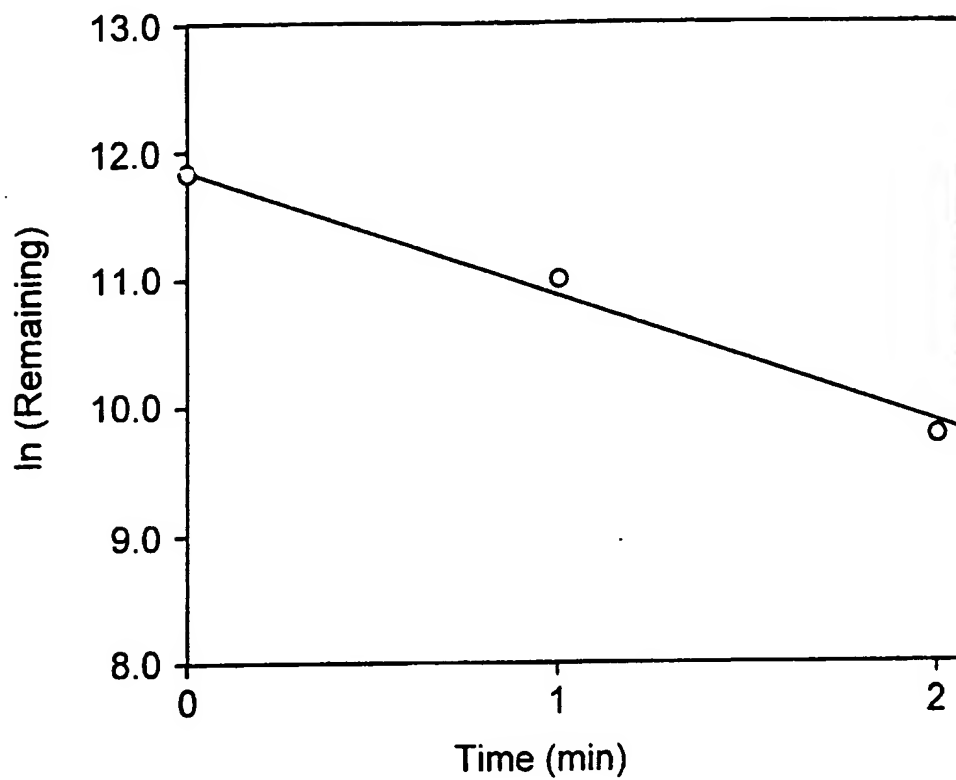


FIG. 15

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$$y = -0.913 \ln x + 12.285$$
$$r = 0.930, t_{1/2} = 0.76 \text{ min}$$

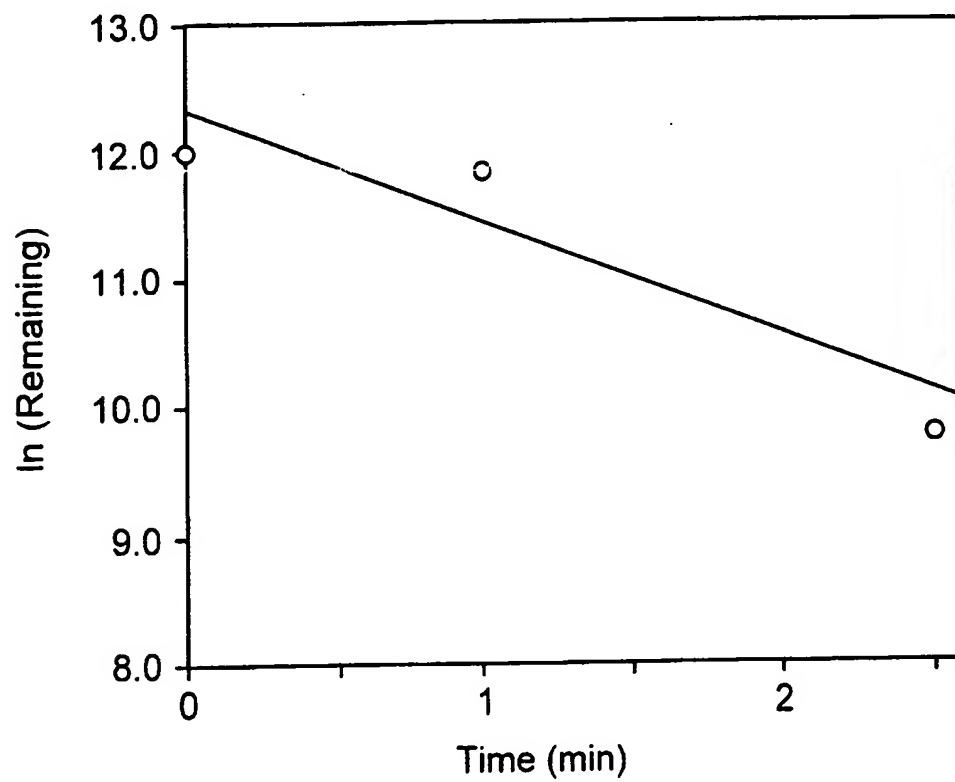


FIG. 16

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$$y = -0.021 \ln x + 12.523$$

$$r = 0.950$$

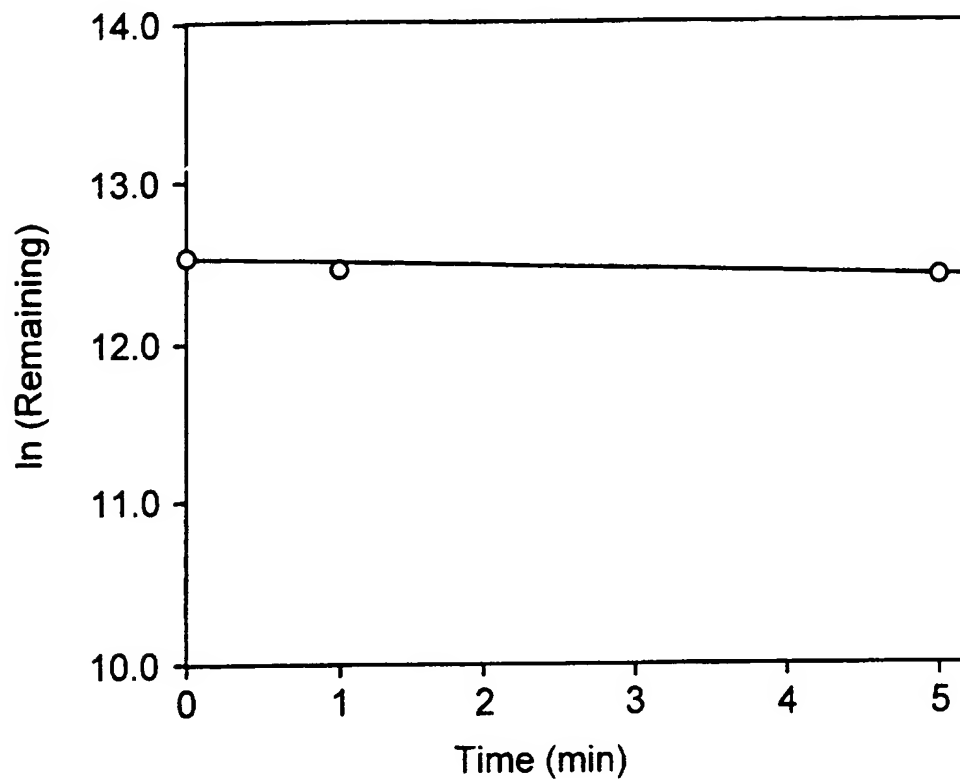


FIG. 17

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$$y = -0.0048 \ln x + 14.510$$

$$r = 0.969$$

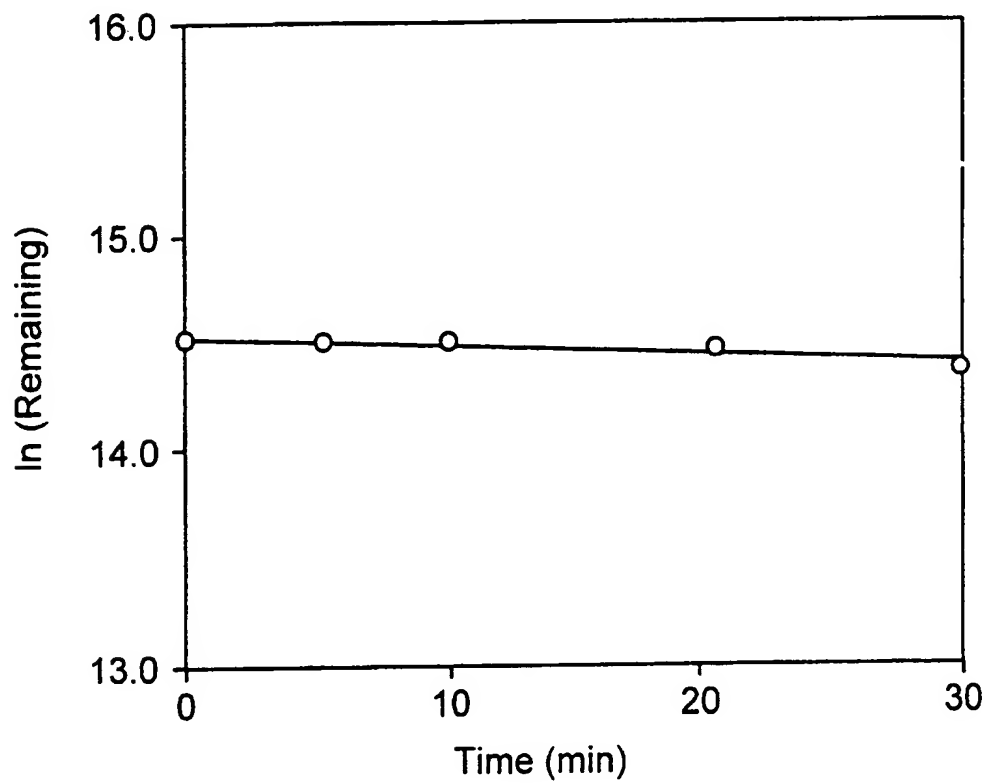


FIG. 18

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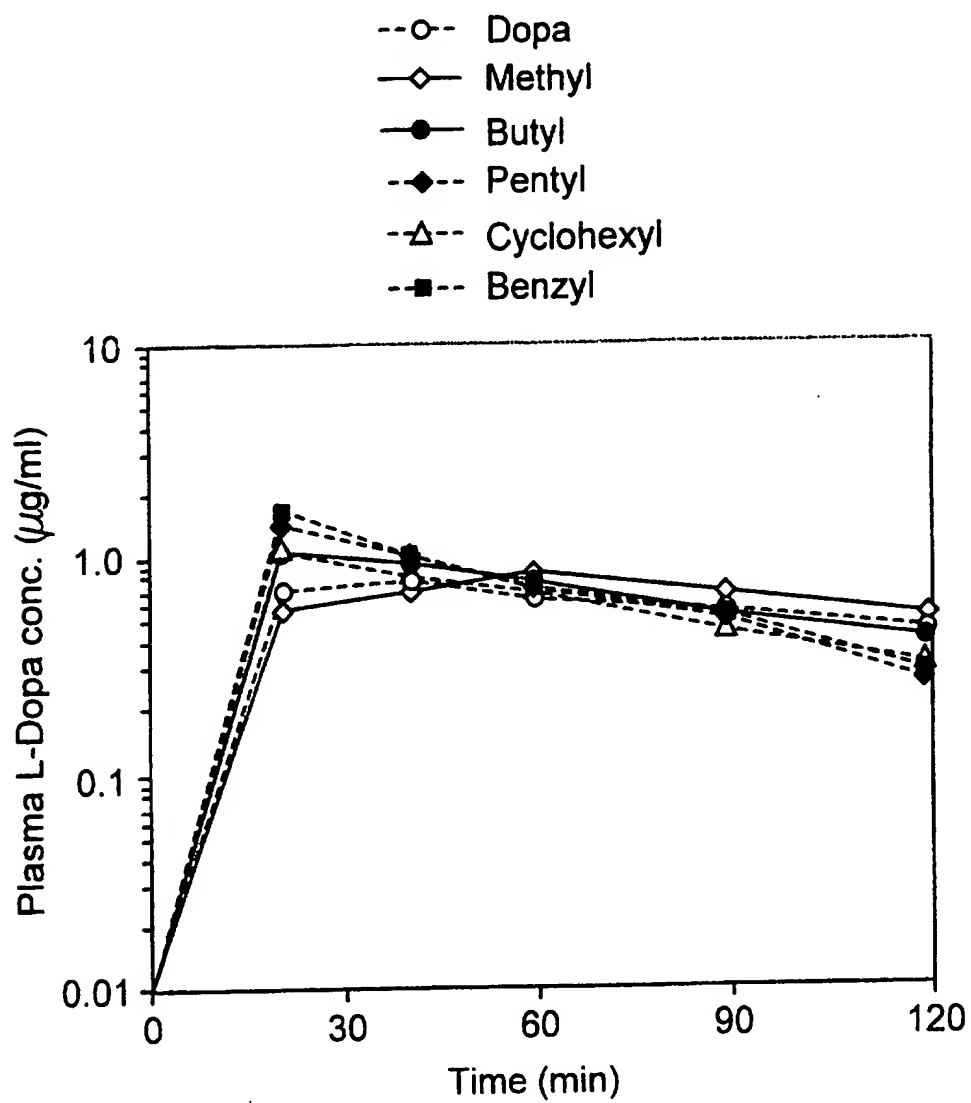


FIG. 19

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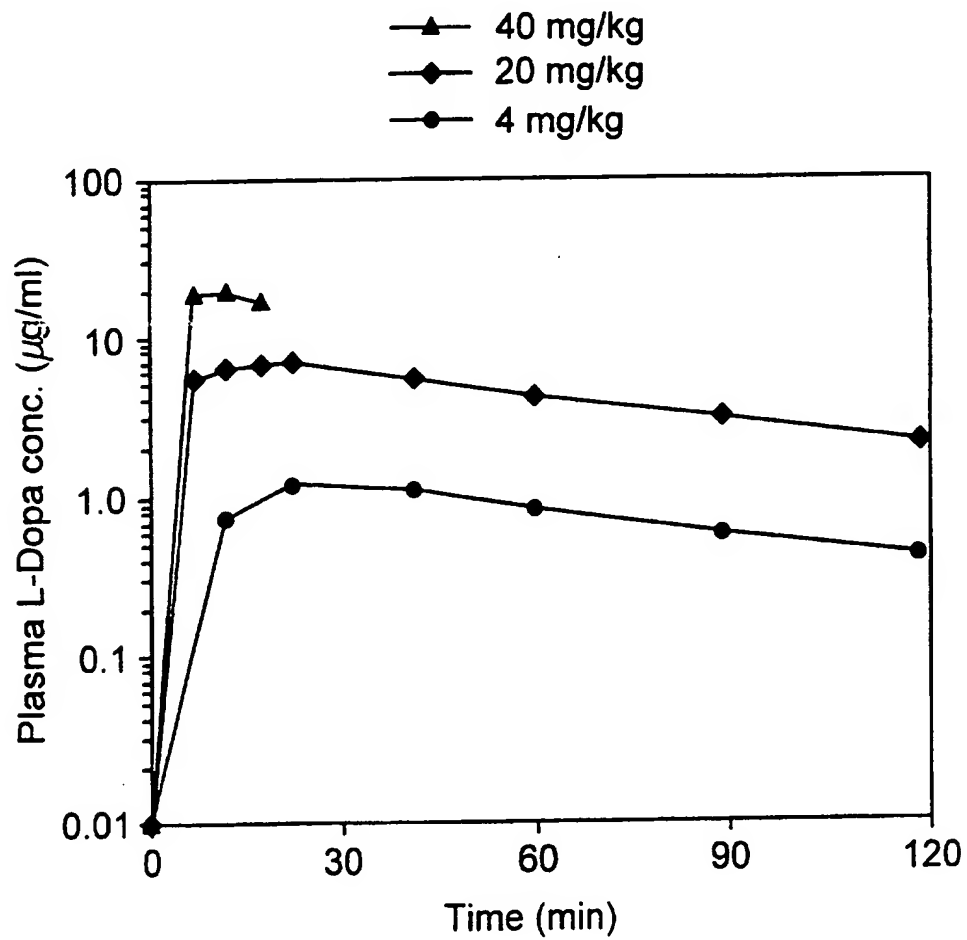


FIG. 20

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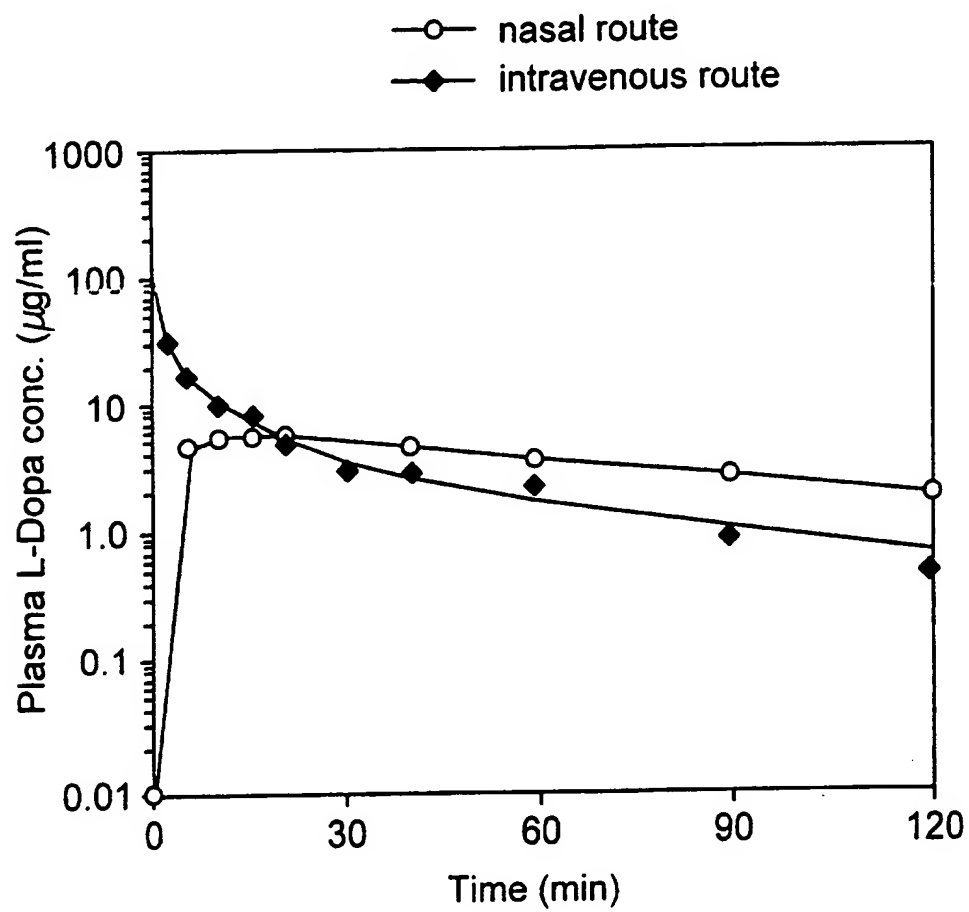


FIG. 21

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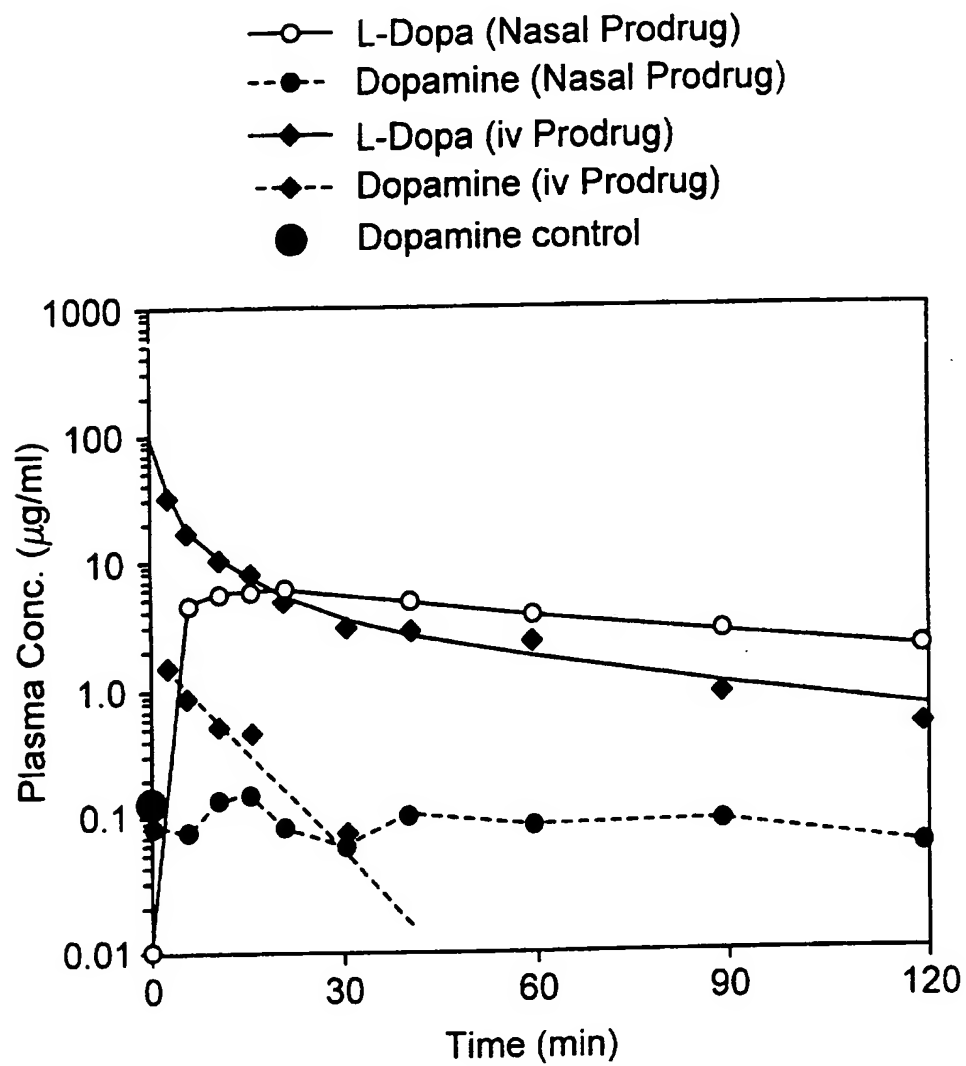


FIG. 22

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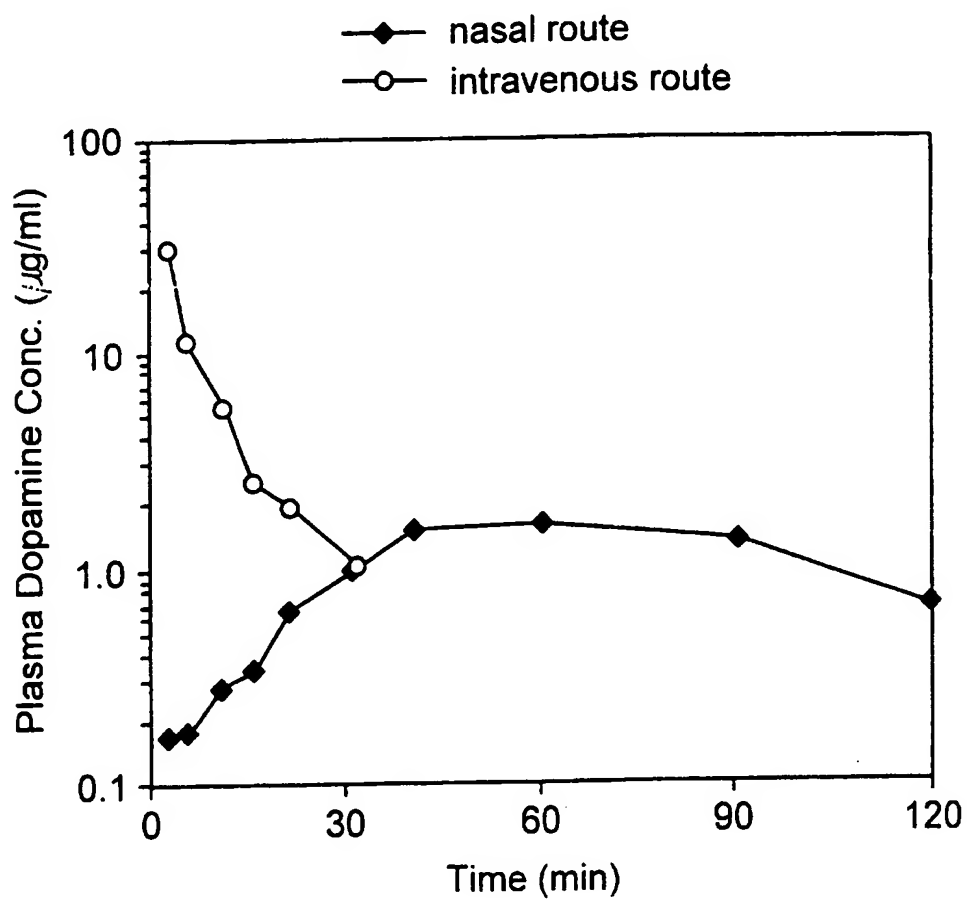


FIG. 23

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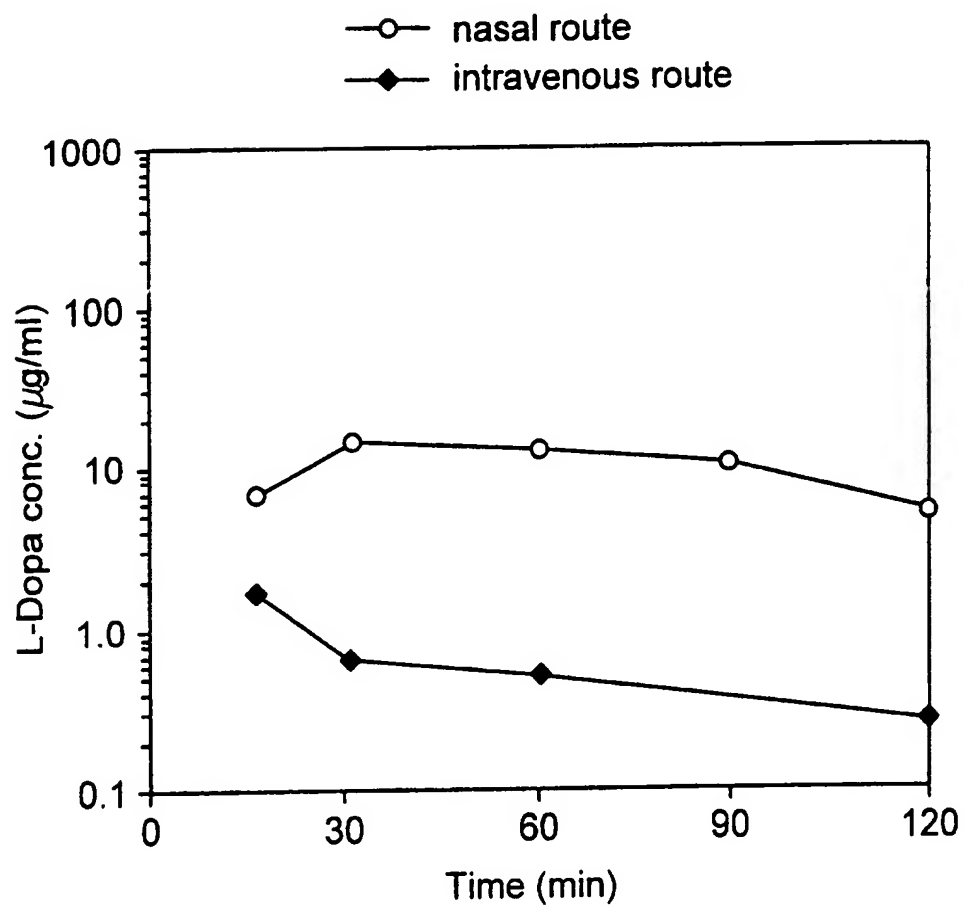


FIG. 24

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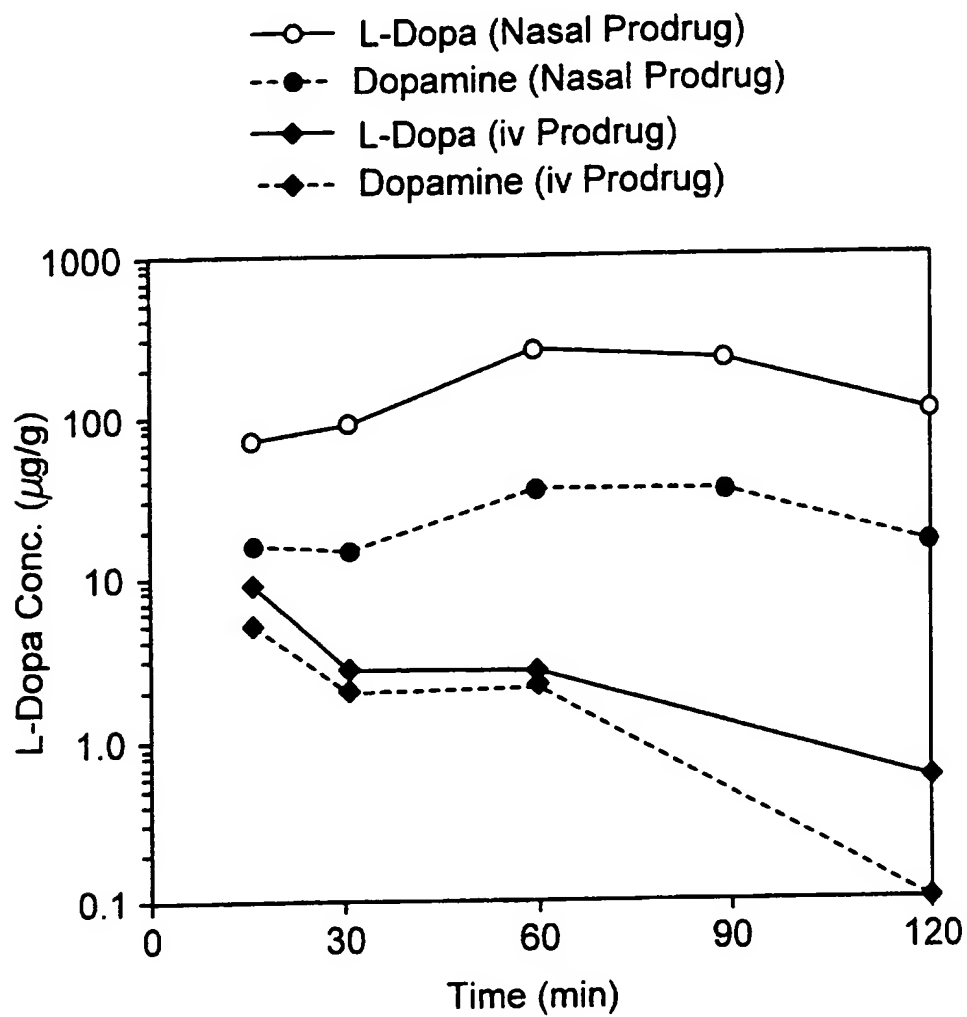


FIG. 25

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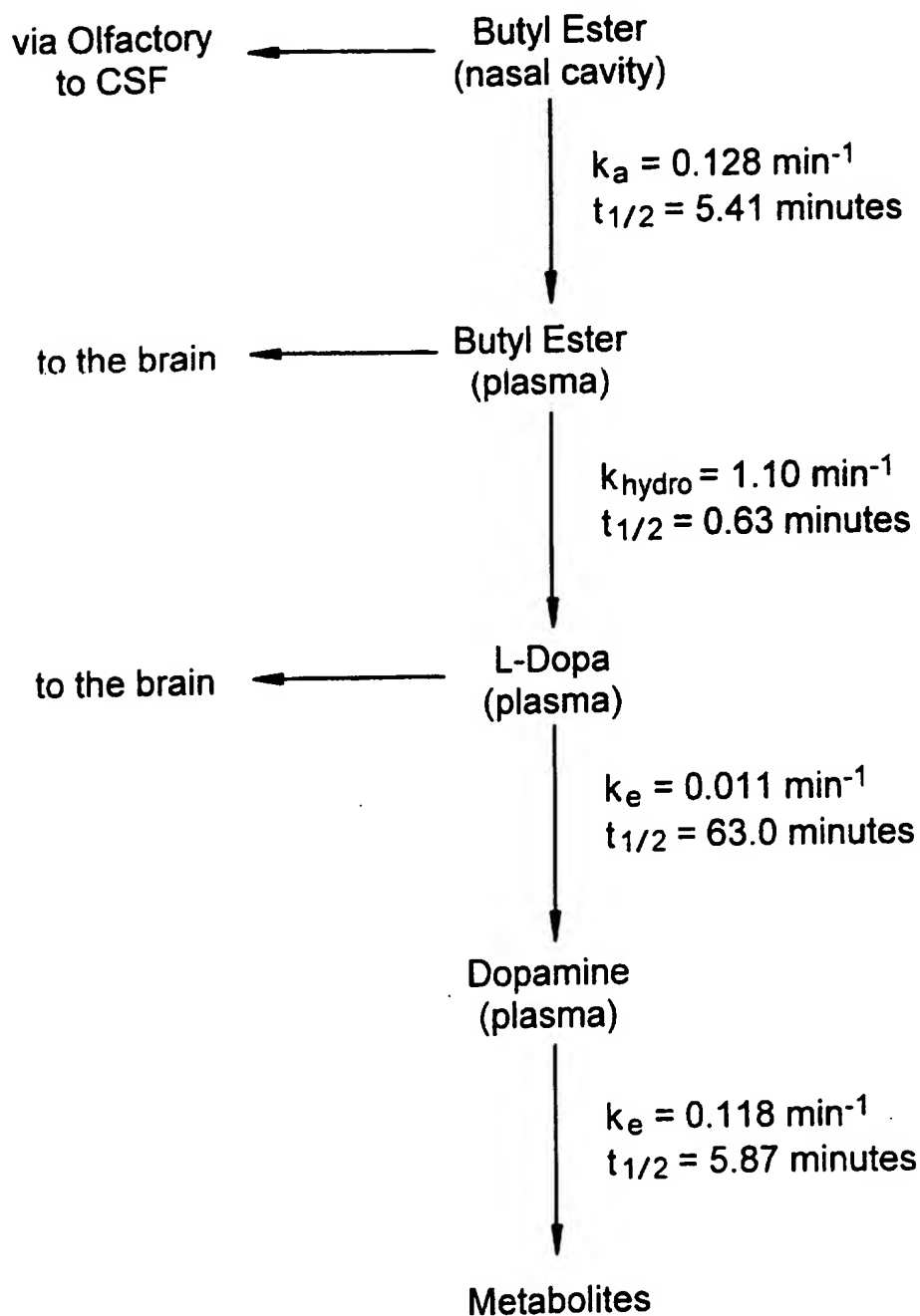


FIG. 26

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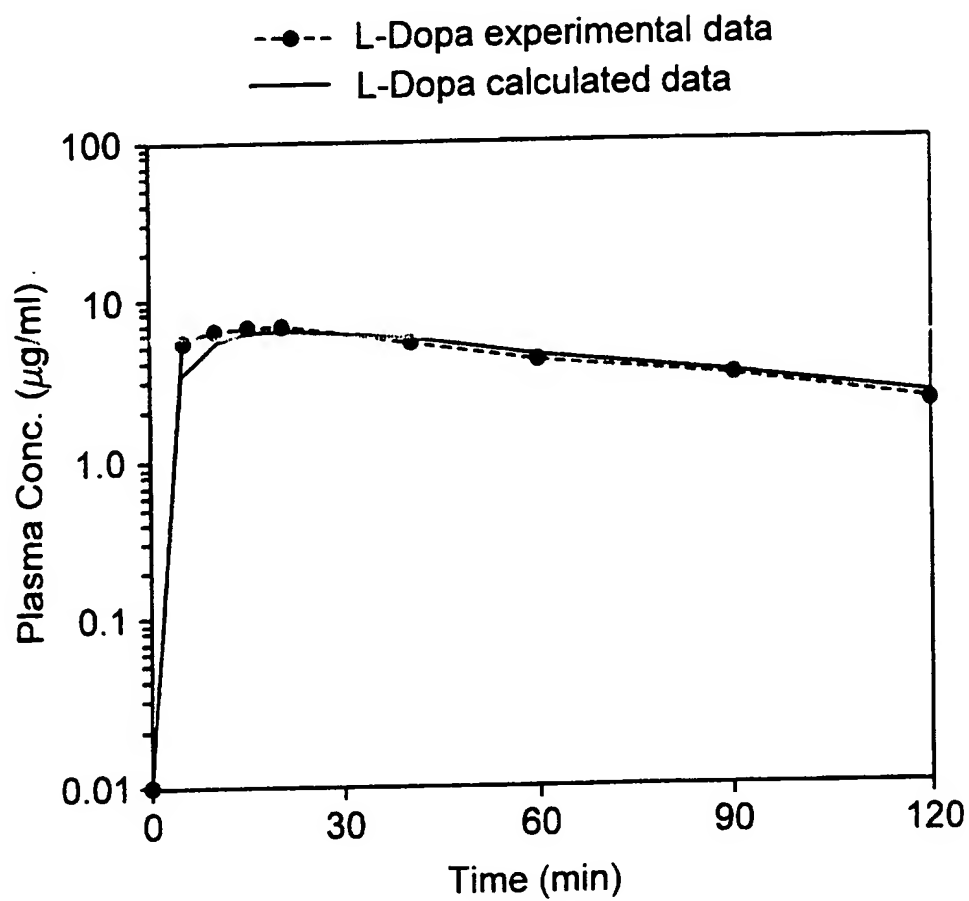


FIG. 27

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/17740

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/24

US CL :514/538

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/538

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS

search terms: L-dopa, dopamine deficiency, Parkinson's Disease, intranasal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,663,349 A (REPTA) 05 MAY 1987, column 1, line 48, column 2, and column 3.	1-18
Y	US 5,354,885 A (MILMAN ET AL) 11 October 1994, column 4, lines 16-20.	1-18
Y	HUANG et al. Mechanism of Nasal Absorption of Drugs II: Absorption of L-Tyrosine and the Effect of Structural Modification on its Absorption. Journal of Pharmaceutical Sciences. December 1985. Vol. 74. No. 12. pages 1298-1301, entire document.	1-18

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

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